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Genetic analysis of the floral initiation process (FLIP) in *Arabidopsis*

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SUMMARY

Within the *Arabidopsis* inflorescence, two distinct developmental phases exist. The early inflorescence phase is characterized by nodes bearing coflorescences and leaves, and the late inflorescence phase by nodes bearing flowers. Four genes, *TERMINAL FLOWER 1, LEAFY, APETALA1* and *APETALA2* are necessary to initiate the switch from formation of early to formation of late inflorescence nodes at the appropriate time. We have investigated the relative roles of these genes in development by isolating and characterizing new alleles of *TERMINAL FLOWER 1, LEAFY* and *APETALA1*, and by constructing double mutants to test gene interactions. We suggest that the *TERMINAL FLOWER 1* gene product is part of a mechanism that controls the timing of phase-switching in *Arabidopsis*. We propose that this mechanism involves factor(s) whose activity changes in response to shoot development and environmental variation. *TERMINAL FLOWER 1* influences phase transitions in *Arabidopsis*, and appears to regulate the timing of expression of *LEAFY, APETALA1* and *APETALA2*. *LEAFY, APETALA1* and *APETALA2* have partially redundant functions in initiating the floral program. In the absence of any one of the three genes, there is a gradual transition from coflorescence to flower-like lateral shoots. This suggests that (1) *LEAFY, APETALA1* and *APETALA2* are required in combination to ensure that the floral program is initiated rapidly and completely and (2) in the absence of one of the three genes, the others are activated slowly in response to the mechanism controlling timing of phase switching. Besides their role in establishing the floral program, phenotypes of flower-like lateral shoots in mutant inflorescences suggest that all three, *LEAFY, APETALA1* and *APETALA2*, influence expression of whorl identity genes. Loss of *LEAFY* results in decreased Class B gene expression, as well as altered expression patterns of Class A and Class C genes. In the absence of either *APETALA2* or *APETALA1*, reproductive organs develop in the perianth whorls, suggesting that both genes should be considered Class A organ identity genes, restricting Class C gene expression to inner whorls.

Key words: *Arabidopsis*, flower initiation, *TERMINAL FLOWER, LEAFY, APETALA*

INTRODUCTION

The growth of any plant shoot occurs through the production of nodes by the shoot apex. During the plant life cycle, the shoot apex proceeds through a series of developmental phases (Poethig, 1990; McDaniel et al., 1992). Each phase is reflected by the changing fate of the organ or lateral meristem borne at the nodes. Within a species, the sequence and timing of developmental phases is remarkably constant. Moreover, the timing of phase switching is responsive to the environment (for example, time to flowering reviewed by Napp-Zinn, 1969). Based on these observations, the control of phase switching requires an environmentally sensitive mechanism that detects developmental time and in response activates appropriate morphological programs.

Development of the *Arabidopsis* shoot can be divided into four phases (Fig. 1), based on node morphology at maturity: (1) juvenile rosette, (2) mature rosette (Medford et al., 1992), (3) early inflorescence, and (4) late inflorescence, (Schultz and Haughn, 1991). Within the rosette, nodes are closely appressed and bear a leaf and a lateral meristem which may develop into an inflorescence. Elongation of internodes (bolting) designates the beginning of the inflorescence. Otherwise, early inflorescence nodes, which produce a leaf subtending a lateral inflorescence (coflorescence, Weberling, 1989), are similar to the late rosette nodes, and the two node types may be considered to occur within a single developmental phase. In the late inflorescence phase, nodes lack leaves and lateral meristems develop as flowers instead of coflorescences.

The most dramatic morphological change within the inflorescence, which requires an alteration of both organ and lateral meristem fate, is the transition from nodes bearing coflorescences with subtending leaves to nodes bearing flowers. Coflorescences are indeterminate shoots which produce several lateral coflorescences before a number of lateral flowers, thus reiterating the inflorescence program (Figs 1, 2). In contrast, flowers are determinate shoots, producing a set of specialized organs in an invariant sequence and arrangement. Thus, the change from coflores-

cence to floral program requires the suppression of internode elongation, the suppression of lateral shoot development, the loss of indeterminate growth, and the initiation of floral-specific organ type and arrangement.

Recent molecular and genetic analysis in both *Arabidopsis* and *Antirrhinum majus* have resulted in a model for the control of floral organ identity (Haughn and Somerville, 1988; Schwarz-Sommer et al., 1990; Bowman et al., 1991; Coen and Meyerowitz, 1991). The two basic tenets of the model are firstly that three gene classes with overlapping fields of expression control whorl identity. Class A genes, such as *APETALA2* (*AP2*) in *Arabidopsis*, act in whorls one and two, Class B genes, such as *PISTILLATA (PI)* and *APETALA3 (AP3)* act in whorls two and three, and Class C genes, such as *AGAMOUS (AG)* act in whorls three and four. Secondly, the model states that the expression of Class A and C genes are mutually exclusive, such that each gene class restricts the expression of the other. Class A activity also appears to influence Class B expression, since carpels can be found in the second and third whorls of strong *AP2* alleles (Kunst et al., 1989; Haughn et al., 1993) and AP3 transcript levels are reduced in some *AP2* alleles (Jack et al., 1992). The down regulation of Class B genes in the absence of *AP2* may be the result of direct regulation by *AP2*, or may be the result of ectopic *AG* expression (Schultz et al., 1991; Jack et al., 1992). Finally, *AP2* function is also required for normal development of the gynoecium (Kunst et al., 1989; Haughn et al., 1993).

Although several genes have been identified as playing a role in the inflorescence phase switch, no comprehensive model for their interactions has yet been proposed. Mutations in *TERMINAL FLOWER 1* (*TFL1*) (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992) result in both an earlier bolting time and an earlier formation of flowers, such that coflorescences are replaced by flowers. Moreover, the total number of inflorescence nodes is extremely reduced, and the apical meristem itself develops into a flower. Mutations in a second gene *LEAFY* (*LFY*) (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992) result in inflorescences in which there is a gradual transition from nodes bearing leaves and coflorescences to nodes bearing flower-like structures. Such a phenotype suggests that *LFY* functions in lateral meristems and subtending organ primordia to repress development of coflorescence and leaf and to activate floral development. A third gene, *APETALA1* (*AP1*) (Irish and Sussex, 1990; Bowman, 1992; Mandel et al., 1992), is considered to function in perianth organ identity, since in Ap1 flowers, leaves subtending flowers develop in place of sepals and petals. As in Lfy, the phenotype becomes less extreme acropetally. Ap1 flowers may also be described as a partial transformation of the floral meristem to a coflorescence meristem, an interpretation which would suggest that like LFY, AP1 is involved in suppressing the coflorescence program. This explanation is consistent with results from double mutant analysis (Huala and Sussex, 1992; Weigel et al., 1992; Shannon and Meeks-Wagner, 1993). A fourth gene, *AP2*, has been considered a Class A organ identity gene, involved predominately in perianth whorl identity (Komaki et al., 1988; Bowman et al., 1989; Kunst et al., 1989). However, there is increasing evidence that it has additional roles; certain alleles of *AP2* may result in flowers that form leaves in the first whorl (Bowman et al., 1989) and develop tertiary meristems under specific conditions (Komaki et al., 1988). Moreover, analysis of Ap1/Ap2 double mutants suggests that the two genes have overlapping functions (Irish and Sussex, 1990; Shannon and Meeks-Wagner, 1993). These observations suggest that, like *AP1*, *AP2* may have some role in activating the floral- and deactivating the coflorescence-program in lateral meristems.

In order to understand further the regulation of inflorescence morphogenesis, we have isolated new alleles of *TFL1*, *LFY* and *AP1*, examined mutant phenotypes under different growth conditions, and constructed double and triple mutants to identify gene interactions. On the basis of this analysis we propose a model for the initiation of flower development. This model accounts for most of the available data, including several previously unexplained aspects of both wild-type and mutant development.

MATERIALS AND METHODS

Plant material

All novel mutant lines described in this analysis were isolated from ethyl methanesulfonate-mutagenized populations of *Arabidopsis* ecotype Columbia. Before being used for analysis, lines were backcrossed to wild type as follows: Tfl1-14, 3 times; Tfl1-11, 2 times; Tfl1-12, 2 times; Tfl1-13, 2 times; Lfy-2, 3 times; Ap1-10, 3 times; Ap1-11, 1 time; Ap1-12; 3 times; Ap1-13, 3 times; Ap1-14, 1 time. Lines used for construction of double mutants were SAS 1-2-6 (homozygous for *ap2-5*, backcrossed three times to wild-type Columbia), SAS 1-3-7 (homozygous for *ap2-6*, backcrossed two times to wild-type Columbia; Kunst et al., 1989), SAS 1-13-0 (heterozygous for *ag-1*), SAS 1-0-0 (homozygous for *ap2-1*), SAS 1-10-0 (homozygous for *ap1-1*; gifts from Maarten Koornneef).

Plants were grown at 22°C under Grow-Lux fluorescent light (Sylvania) on Tera-lite Redi-earth prepared by W.R. Grace & Co. Canada Ltd., Ajax, Ontario, Canada. Plants were exposed to three photoperiodic regimes: continuous light (CL) - continuous illumination, 100-120 μE m^{-2} $second^{-1}$ PAR; long day (LD) - 16 hours illumination, 120-150 μE m^{-2} sec^{-1} PAR; short day (SD) - 10 hours illumination, 150-180 μE m^{-2} sec^{-1} PAR. Plants grown under shorter day conditions but with equivalent light intensities were unhealthy, likely as a result of reduced photosynthates; therefore, we increased the light intensity as the daylength decreased.

Analysis of shoot structure

Approximately equal numbers of seeds were sown in 4 inch diameter pots and kept for 3 days at 4°C to synchronize germination. Time to bolting (BT) was counted as the number of days from removal from vernalization to the elongation of the inflorescence shoot to 1 cm. Number of rosette nodes was counted at this time.

At least 50 2° shoots were taken from plants of each genotype at various positions within the inflorescence for morphological characterization using the dissecting microscope and scanning electron microscope. Samples were prepared for SEM as described in Schultz et al., 1991.

Construction of double and triple mutant lines

The doubly and triply mutant lines listed in Table 1 were isolated from F_2 populations generated by cross-pollinating parental lines homozygous for individual mutations. In the case of the *Ag-1* line, which is both male and female sterile, a heterozygote was used. The F_2 frequency observed for each of the double mutants was consistent with that expected on the basis of Mendelian segregation

(Table 1). In addition, unless both parental phenotypes were self sterile (eg. Lfy-1, Ag-1) progeny of several F_2 plants with a parental phenotype were examined. The appearance of the parental phenotype and the novel phenotype in a 3:1 ratio among the progeny of two thirds of the selected F_2 plants (Table 1) supported the conclusion that the novel phenotype represents the double or triple mutant.

RESULTS

Terminology

The inflorescence structure of *Arabidopsis* is a potentially infinite series of branching shoots since each indeterminate shoot has the capacity to initiate additional indeterminate shoots. We will refer to the shoot produced by the main apical meristem as primary (1°). We will divide the 1° inflorescence into two halves, the basal 1° inflorescence, and the upper 1° inflorescence. Any lateral shoot developing from the primary shoot will be referred to as secondary (2°) and one developing from a 2° shoot will be referred to as tertiary (3°) (Fig. 1).

Although wild type 2° shoots are either coflorescences or flowers, many of the mutants we describe here have 2° shoots with characteristics of both coflorescences and flowers (Fig. 2). Because any of the shoots may end in a fused pistil-like structure and few of the shoots have completely normal floral organs, presence of floral organs and determinacy cannot be used to distinguish shoot types. To simplify the description, unless otherwise stated, we will refer to shoots as coflorescence-like if they have 3° shoots and internode elongation, and as flower-like if they have neither 3° shoots nor internode elongation. However, it is important to note that the distinction between coflorescence-like and flower-like is artificial, since a complete spectrum of shoots with varying degrees of floral and coflorescence features can be observed.

The nomenclature of leafy organs occurring within the *Arabidopsis* shoot needs to be clarified. Leaves formed on the 1° shoot early in development and not separated by internode elongation are consistently called rosette leaves. Leaves formed on the 1° shoot and separated by internode elongation have been called either bracts (Schultz and Haughn, 1991) or cauline leaves (Huala and Sussex, 1992; Weigel et al., 1992). Leaves formed on 2° shoots have consistently been called bracts (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992). An analysis of shape and epidermal cell types in leaves (Martinez-Zapater et al., 1993) indicates a continuum of gradual change occurs throughout shoot development, and therefore suggests that distinctions among leafy organs is somewhat arbitrary. Thus, in the following discussion, we will refer to all leafy organs simply as leaves.

Terminal flower 1

Four allelic lines having similar phenotypes, one of which we have described under the name *TERMINATOR* (Schultz and Haughn, 1991), were isolated from EMS mutagenized *Arabidopsis* populations of the Columbia ecotype. Except for the early-flowering aspect of the phenotype, which is partially dominant, all phenotypic characteristics are recessive. Similarities between the Terminator and Terminal Flower 1 phenotypes (Tfl11; Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992, Shannon and Meeks-Wagner, 1993) led to complementation analyses that confirmed that

Table 1. Construction of doubly and triply mutant lines

Parental phenotypes	Expected F_2 ratio	Number of plants[a]	χ^2	*P*	Expected F_3 ratio[b]	Number of plants[c]	χ^2	*P*
Lfy-1, Tfl1-14[d]	9:3:3:1	68	1.34	> 0.5	3:1	256	0.75	> 0.25
Lfy-1, Ap1-1	9:3:4[e]	332	2.32	> 0.25	3:1	57	0.47	> 0.5
Lfy-1, Ap1-10	9:3:4	126	0.87	> 0.5				
Lfy-1, Ap2-1	9:3:4	61	0.41	> 0.75	3:1	38	0.04	> 0.75
Lfy-1, Ap2-6	9:3:4	145	0.66	> 0.5	3:1	15	0.02	> 0.75
Lfy-1, Wt[f]	9:3:3:1	113	1.64	> 0.5				
Lfy-2, Tfl1-14	9:3:3:1	162	3.73	> 0.25	3:1	48	5.44	> 0.025
Lfy-2, Ap1-1	9:3:3:1	70	1.11	> 0.75	3:1	12	0.36	> 0.5
Lfy-2, Ap1-10	9:3:3:1	65	1.38	> 0.5	3:1	66	0.02	> 0.75
Lfy-2, Ap1-13	9:3:3:1	110	6.75	> 0.05	3:1	54	0.025	> 0.75
Lfy-2, Ap2-1	9:3:3:1	119	1.67	> 0.5	3:1	40	0.53	> 0.25
Lfy-2, Ap2-6	9:3:3:1	131	4.83	> 0.1	3:1	53	0.03	> 0.75
Lfy-2, Wt[f]	9:3:3:1	49	1.83	> 0.5	3:1	35	0.24	> 0.5
Tfl1-14, Ap1-1	9:3:3:1	283	1.05	> 0.75[g]				
Tfl1-14, Ap1-10	9:3:3:1	44	0.54	> 0.9	3:1	13	0.23	> 0.5
Tfl1-14, Ap1-13	9:3:3:1	120	1.96	> 0.5				
Tfl1-14, Ap1-14	9:3:3:1	156	0.44	> 0.9				
Lfy-1/Tfl1-14, Ap1-1/Tfl1-14	9:3:3:1	23	7.2	> 0.05	3:1	55	2.25	> 0.1

[a] Number of plants scored in the F_2 population

[b] F_3 populations were generated by allowing F_2 plants of parental phenotype to self-pollinate, as described in Materials and Methods

[c] Number of plants scored in the F_3 population

[d] Unless otherwise stated, all parents were homozygous for the mutant allele

[e] Because of the variability of the Lfy-1 phenotype, doubly mutant plants in the F_2 population cannot be distinguished from the single mutant with certainty, resulting in a 9:3:4 ratio

[f] Parents having wild-type phenotype (Wt) were heterozygous for *ag-1*

[g] Putative Tfl1-14/Ap1-1 double mutants were confirmed by test-crosses to Tfl1-14 parents

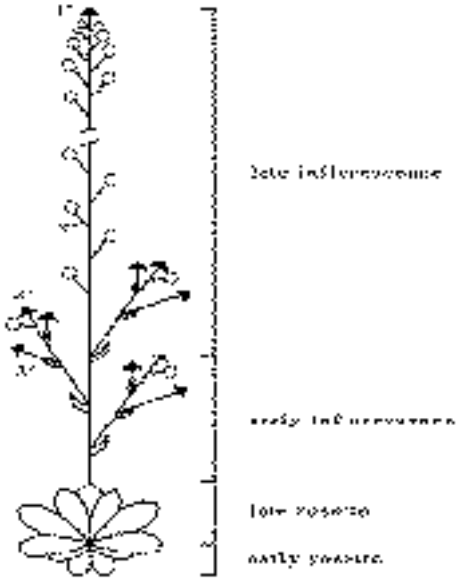


Fig. 1. Diagrammatic representation of wild-type *Arabidopsis* shoot morphology. Four regions of the 1° shoot having distinct nodal morphology are shown. Shoots are designated as 1°, 2°, or 3° depending on their origin, as described in the text. = rosette leaf; = inflorescence leaf; ⟶ = inflorescence shoot (1° or 2°); —○ = flower.

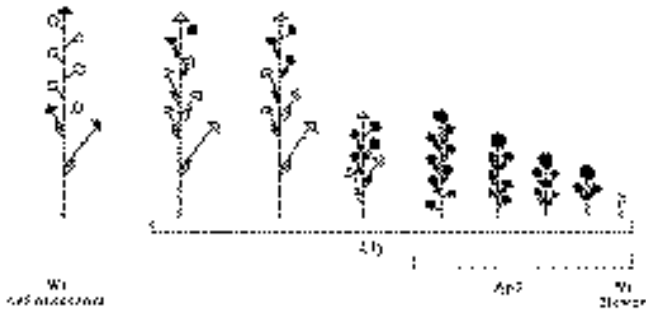


Fig. 2. Diagrammatic representation of 2° shoot types formed in Wt, Lfy and Ap1 inflorescences. Organ and 3° shoot type and arrangement become more flower-like from left to right (see Figs 4-6 for more detail). Symbols as in Fig. 1 as well as: = carpelloid organ; —▷ = inflorescence-like shoot (1° or 2°); —● = flower-like shoot.

both phenotypes are due to alleles of the same gene (data not shown). Consequently, we have renamed our alleles *tfl1-11*, *tfl1-12*, *tfl1-13* and *tfl1-14*. Because of the similarity of the four mutant phenotypes (Table 2), we will describe in detail only that of Tfl1-14.

The Tfl1-14 phenotype is similar to strong alleles described previously (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). Like other alleles in Columbia ecotype (Shannon and Meeks Wagner, 1991), plants homozygous for *tfl1-14* have an earlier BT than wild type under continuous light, and a correspondingly fewer number of rosette leaves (Table 2). The early flowering Landsberg *erecta* genetic background (Table 2) may have obscured a similar early BT in other *tfl1* alleles (Alvarez et al., 1992).

Tfl1 plants develop a highly modified inflorescence (Fig. 3). Compared to wild type, Tfl1-14 inflorescences produce slightly fewer leaf-bearing nodes usually having flower-like 2° shoots rather than coflorescences. Studies of these 2° shoots in early stages of development reveal that organs are initiated in the whorled pattern typical of flowers (data not shown). Most often (63%), flowers develop that are wild type except for an elongated pedicel. Occasionally, 2° shoots are coflorescence-like such that a leaf-like organ develops on the pedicel below the flower (32%), or a cluster of two to three flowers develop (5%). Before initiation of 2° shoots ceases, the inflorescence forms 1-2 flowers that are not subtended by any leafy organ. Thus, the *terminal flower 1* mutation reduces the number of nodes produced in each phase of shoot development.

The terminal region of the Tfl1-14 1° inflorescence may consist of a single flower (42%) or of 2-3 clustered flowers separated by little internode elongation (58%). Examination of early developmental stages of this terminal region suggests that the apical meristem itself develops as the terminal flower(s) (data not shown; Shannon and Meeks-Wagner, 1991); therefore, the Tfl1-14 inflorescence may be described as determinate. While the 2° flowers produced in the Tfl1-14 inflorescences are completely wild type both in organ type and arrangement, organs in the three outer whorls of the 1° flower may be missing or may be mosaic organs (Alvarez et al., 1992; Shannon and Meeks-Wagner, 1991; Schultz and Haughn, data not shown). However, in all flowers examined, the gynoecium appears to be wild type. The phenotype of these terminal flowers is similar to a weak Lfy phenotype (this study, Huala and Sussex, 1992; Weigel et al., 1992).

Photoperiod affects Tfl1

Time to formation of flowers is highly influenced by photoperiodic conditions. As daylength decreases, the number of nodes in each developmental phase increases. Thus, wild-type plants grown under short day conditions have increased numbers of leaf-bearing nodes (rosette and early inflorescence) compared to plants grown under CL (Fig. 3 and Table 2, Koornneef et al., 1991). In order to determine if our alleles, like those of Shannon and Meeks-Wagner (1991), retain daylength sensitivity, we compared the four new Tfl1 phenotypes (Table 2) under three photoperiodic conditions, CL, LD and SD. As described in the methods, it was necessary to change light intensity as well as the photoperiod, but we will refer to both changes simply as photoperiodic changes. Under LD (data not shown), BT is increased, plants produce more rosette nodes, and 2° shoots show more coflorescence-like tendencies than under CL. Under SD conditions the Tfl1 phenotype is more obviously altered (Fig. 3 and Table 2, Shannon and Meeks-Wagner, 1991). BT is significantly longer, more rosette leaves are formed, and the first nodes of the inflorescence bear coflorescences, followed by a greater number of flowers than in CL. As under CL, both 1° and 2° inflorescence shoots terminate in a group of closely appressed flowers. Quite often (45%), the 1° meristem senesces before 2° shoots of the upper inflorescence have matured beyond the primordial stage. Although

Table 2. Comparison of shoot morphology of various phenotypes grown under (A) Continuous Light and (B) Short Day conditions

Phenotype	Number	BT	RL	IL	CF	F+ 3°	F
(A) Continuous Light							
WT (COL)	29	24.0±2.6[a]	11.0±1.2	3.1±0.8	3.1±0.8	0	28.0±4.6
Tfl1-14	24	16.0±1.1[b]	7.4±0.7[b]	1.8±0.9[b]	0.4±0.6[b]	0	2.5±1.4[b]
Tfl1-11	20	16.0±1.6[b]	9.0±0.9[b]	0.8±0.6[b]	0.9±0.7[b]	0	2.0±1.2[b]
Tfl1-12	11	17.0±2.3[b]	9.0±1.5[b]	0.8±0.7[b]	1.1±0.8[b]	0	1.7±1.5[b]
Tfl1-14/Lfy-2	21	13.0±1.0[c,e]	7.8±0.7[e]	3.6±0.6[b,c,e]	3.4±0.9[c,e]	0	3.8±1.0[b,c]
Tfl1-14/Lfy-1	10	15.0±1.1[c,d]	7.8±0.8[d]	8.2±1.5[b,c,d]	3.9±0.8[c,d]	0	6.4±1.4[b]
Ap1-12	18	17.0±1.9[b]	8.2±1.5[b]	2.6±0.7[b]	2.6±0.7[b]	7.7±3.2[b]	9.4±2.7[b]
Ap1-13	15	20.0±2.7[b]	8.8±1.3[b]	2.4±0.5[b]	2.4±0.5[b]	3.1±1.9[b]	11.0±3.4[b]
Ap1-10	11	18.0±2.1[b]	7.9±1.2[b]	1.9±0.6[b]	11.0±2.2[b]	2.1±2.2[b]	2.8±3.1[b]
Lfy-2	29	19.0±4.4[b]	9.7±2.1	5.3±2.3[b]	9.7±3.2[b]	2.9±2.2[b]	10.0±5.9[b]
Lfy-1	9	20.0±1.6[b]	9.5±1.5	15.0±5.7[b]	18.0±3.0[b]	0	8.4±9.5[b]
WT (LER)	14	18.0±1.0	6.9±0.5	1.9±0.3	1.9±0.3	0	18.0±3.5
Ap1-1	17	18.0±1.7	6.0±0.5[f]	2.0±0.4	5.4±2.3[f]	9.4±3.6[b]	0[f]
Ap2-1	20	20±2.1[f]	7.8±1.0[f]	2.0±0.3	2.0±0.3	0	21.0±3.6[f]
(B) Short days							
WT (COL)	27	66.0±6.5	29.0±4.2	10.0±2.6	10.0±2.3	0	26.0±6.6
Tfl1-14	11	34.0±4.2[b]	14.0±2.6[b]	2.1±0.7[b]	2.2±0.8[b]	0	19.0±9.7[b]
Tfl1-11	14	51.0±9.2[b]	23.0±4.8[b]	5.9±2.5[b]	5.8±2.8[b]	0	27.0±7.2
Tfl1-12	5	45.0±3.7[b]	23.0±2.1[b]	5.3±2.2[b]	5.3±2.2[b]	0	27.0±15.0
Tfl1-14/Lfy-2	17	40.0±8.1[b]	17.0±4.8[b,e]	16.0±5.6[b,c,e]	33.0±8.0[b,c,e]	0	0[b,c]
Tfl1-14/Lfy-1	8	30.0±10.0[b,d]	14.0±8.6[b,d]	19.0±5.7[b,c]	23.0±10.0[b,c]	0	2.7±5.2[b,c]
Ap1-12	15	57.0±12.0[b]	24.0±8.6[b]	7.7±3.0[b]	13.0±6.7	1.8±2.7[b]	15.0±5.8[b]
Ap1-13	17	66.0±10.0	26.0±12.0	9.2±2.1	18.0±8.0[b]	1.4±3.0[b]	9.8±8.4[b]
Ap1-10	9	50.0±4.9[b]	29.0±4.0	8.8±1.3	29.0±9.9[b]	0	0[b]
Lfy-2	9	44.0±3.5[b]	29.0±8.0	54.0±11.0[b]	55.0±12.0[b]	0	0[b]
Lfy-1	7	54.0±4.5[b]	26.0±5.8	24.0±7.1[b]	30.0±5.3[b]	0	0[b]
WT (LER)	13	47.0±5.3	19.0±3.9	6.8±1.3	6.8±1.3	0	30.0±4.2
Ap1-1	5	39.0±5.9[f]	13.0±2.9[f]	4.0±1.4[f]	21.0±5.1[f]	0	0[f]
Ap2-1	15	58.0±8.4[f]	15.0±1.9[f]	5.1±1.6[f]	5.8±1.6	2.8±3.2[b]	23.0±6.1[f]

Number = number of plants scored
BT = bolting time
RL = rosette leaves
IL = inflorescence leaves
CF = coflorescences
F+3° = flowers with 3° mersitems but no elongation
F = flowers
[a] Values given are n±SD
[b] Significantly different from Wt (Col) at the 0.05 level
[c] Significantly different from Tfl1-14 at the 0.05 level
[d] Significantly different from Lfy-1 at the 0.05 level
[e] Significantly different from Lfy-2 at the 0.05 level
[f] Significantly different from Wt (Ler) at the 0.05 level

more frequent in *tfl1* alleles, this premature senescence occurs frequently in all genotypes examined (for example, 29% of wild-type plants).

LEAFY

A number of recessive alleles of the gene *LFY* have previously been described (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992). The phenotypes of the alleles are not identical, but all result in a transformation of floral nodes to coflorescence and leaf bearing nodes. Here we describe in detail the phenotype of a very weak *LFY* allele, *lfy-2*.

Lfy-2

Plants homozygous for the *lfy-2* allele bolt slightly earlier than wild type under CL conditions (Fig. 3 and Table 2), but with no decrease in the number of rosette nodes. As do other Lfy phenotypes (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992), Lfy-2 varies as one proceeds acropetally through the inflorescence, such that basal nodes bear both a leaf and a coflorescence-like 2° shoot, nodes from the mid-inflorescence bear only a coflorescence-like 2° shoot, and upper nodes bear flower-like 2° shoots (Table 2, Figs 2, 3). The 1° inflorescence of both Lfy-1 and Lfy-2 ends in a number of partially fused carpelloid organs (Fig. 4F), as described by Huala and Sussex (1992).

Unlike wild-type coflorescences and as in other Lfy phenotypes (Huala and Sussex, 1992, Schultz and Haughn, data not shown), all Lfy-2 coflorescence-like 2° shoots end in a number of carpel-like organs. Early in the development of these 2° shoots, organs and 3° meristems are initiated in the spiral phyllotaxy typical of wild-type coflorescences (Fig. 4A). Later organs produced by the same 2° shoots are initiated in a pattern which is somewhat more whorled (Fig. 4B,C). As they mature, these organs often overgrow the apical meristem, develop carpel cell types, and partially fuse to one another (Fig. 4D) to form a pistil-like structure (Fig. 4E).

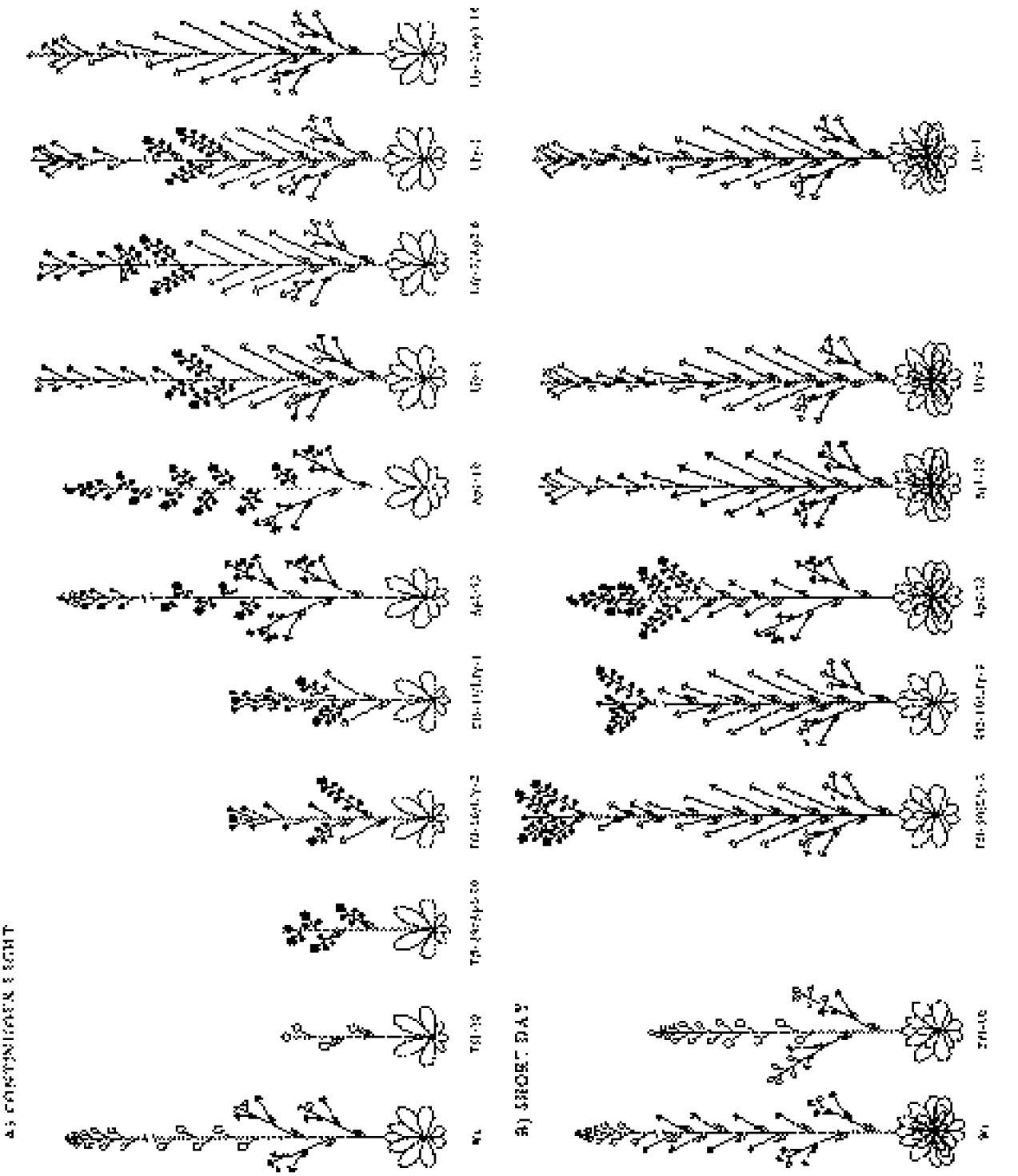


Fig. 3. Diagrammatic representation of the shoot morphology of various *Arabidopsis* genotypes, grown under continuous light (A) and short day (B) conditions. For clarity, the structures of 2° shoots have been simplified (see Figs 2, 4–8, 10 for more detail). Symbols as in Figs 1 and 2.

As one proceeds acropetally in the inflorescence, organ type and arrangement in 2° meristems becomes increasingly like wild-type flowers (Fig. 5). As in wild type, four organs are typically initiated in the outermost whorl, and only rarely are they associated with 3° shoots (Fig. 5A). These may develop into sepal or sepal-carpel intermediate organs (Table 3, Fig. 5F,G). Numbers of primordia initiated in second and third whorls are variable, and the pattern of initiation often deviates from wild type (Fig. 5B-D). Although we examined a large number of flowers, no consistent pattern of primordial initiation or number was evident. Because the fate of these organs is also variable (Table 3 and Fig. 5E-G), assignment of organs to either second or third whorl is inconclusive (Table 3). Most frequently, a normal gynoecial cylinder develops in the fourth whorl (Fig. 5B-D,F-G). Infrequently, the fourth whorl may consist of 3 or 4 fused carpels, or a number of unfused carpels or carpel-stamen intermediate organs (Fig. 5H).

Photoperiod affects Lfy

The Lfy phenotype is known to be affected by photoperiodic conditions (Huala and Sussex, 1992); therefore, we grew both Lfy-1 and Lfy-2 under CL, LD and SD conditions. Like wild type, Lfy plants have both an increased BT and a greater number of rosette nodes under LD compared to CL (data not shown), although the inflorescence phenotype is not significantly different from that seen under CL (Huala and Sussex, 1992). Under SD, the phenotypes of the strong Lfy-1 and the weak Lfy-2 are indistinguishable (Fig. 3 and Table 2). As under CL, both alleles

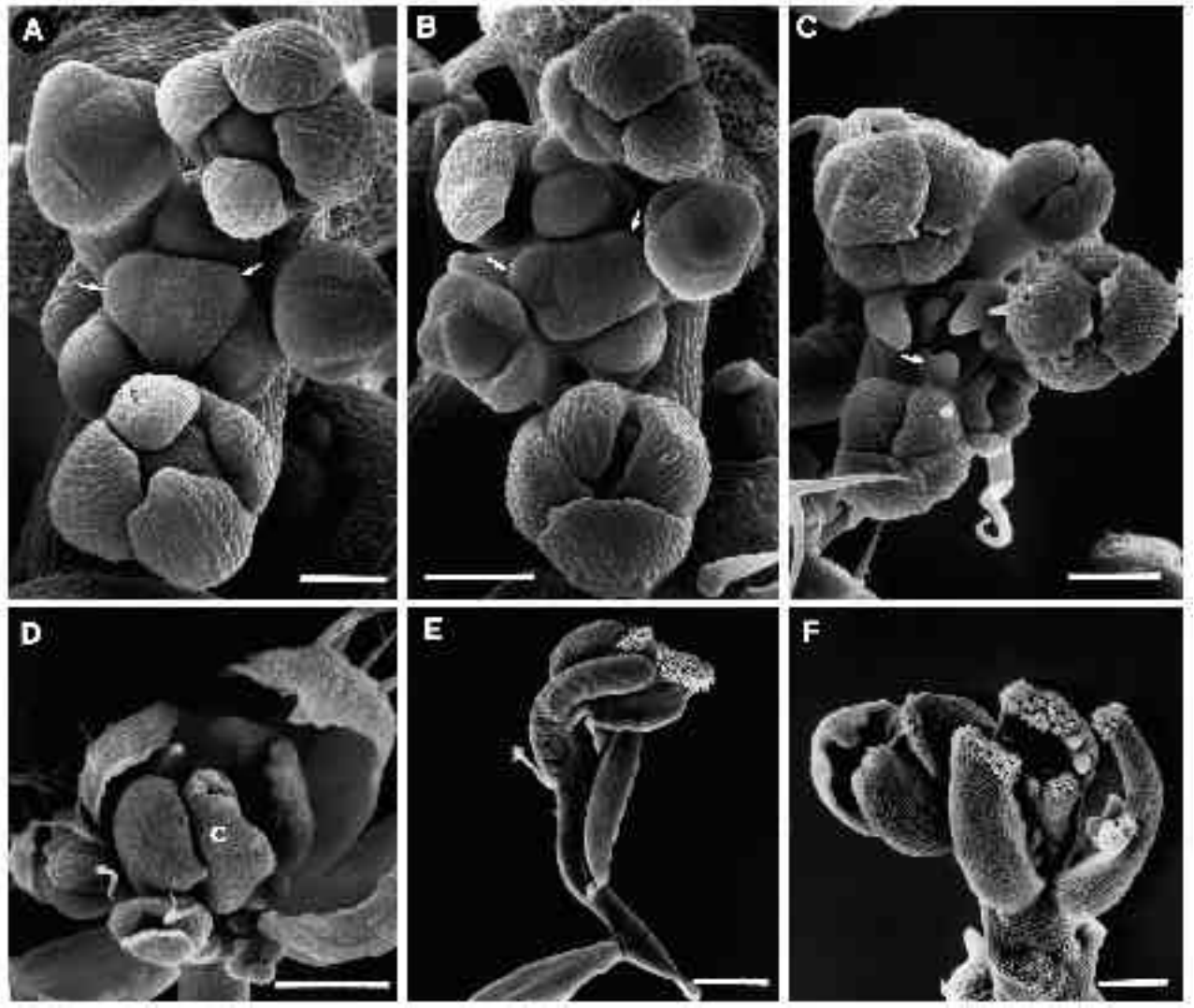


Fig. 4. Scanning electron micrographs illustrating the development of Lfy-2 2° and 1° inflorescence meristems. (A) Early developmental stage of 2° meristem from the basal inflorescence. 3° primordia (arrows) are initiated in spiral phyllotaxy. (B) Later developmental stage of 2° meristem from the basal inflorescence. Primordia are no longer initiated spirally, but rather opposite one another (arrows). (C) Later developmental stage of 2° meristem from the basal inflorescence. 3° primordia (arrow) develop into organ rather than shoot meristems, and overgrow the 2° meristem. (D) Late developmental stage of 2° shoot from basal inflorescence. 3° organs have developed cell types typical of carpels (c), and have fused to form pistil-like structures. (E) Mature pistil-like terminal structure of a 2° shoot from basal inflorescence. (F) Pistil-like terminal structure of 1° meristem. Bars, 50 μm in A and B; 100 μm in C, D and F; 500 μm in E.

bolt slightly earlier than wild type, but with no fewer rosette nodes. They then produce an inflorescence that consists only of leaves subtending coflorescences (Fig. 3).

APETALA1

The phenotype of only a single Ap1 (Ap1-1) mutant has been described in detail (Irish and Sussex, 1990). We have isolated five new *AP1* mutants from EMS mutagenized Columbia populations. Segregation and complementation analyses (data not shown) suggest that all five phenotypes are the result of recessive alleles of the *AP1* gene (data not shown). To distinguish our alleles from others newly isolated (Mandel et al., 1992; Bowman, personal communication), we have designated the alleles *ap1-10*, *ap1-11*, *ap1-12*, *ap1-13*, *ap1-14*. Because Ap1-12, -13 and -14 lines were isolated from a single population, they may not represent independent mutations. All five phenotypes are quite distinct from Ap1-1.

Under CL, plants homozygous for any *ap1* allele flower slightly earlier than wild type, with a correspondingly reduced number of rosette nodes, and then produce a slightly reduced number of coflorescences subtended by leaves (Table 2). The fate of early inflorescence nodes is not altered by *AP1* mutations; therefore in the following analysis we have considered only 2° shoots formed in the late inflorescence phase. As in Lfy, the phenotype of 2° shoots in Ap1 inflorescences are more coflorescence-like in the basal than the upper inflorescence. In addition, those 2° shoots in upper nodes have homeotic conversions, which suggest changes in organ identity gene expression.

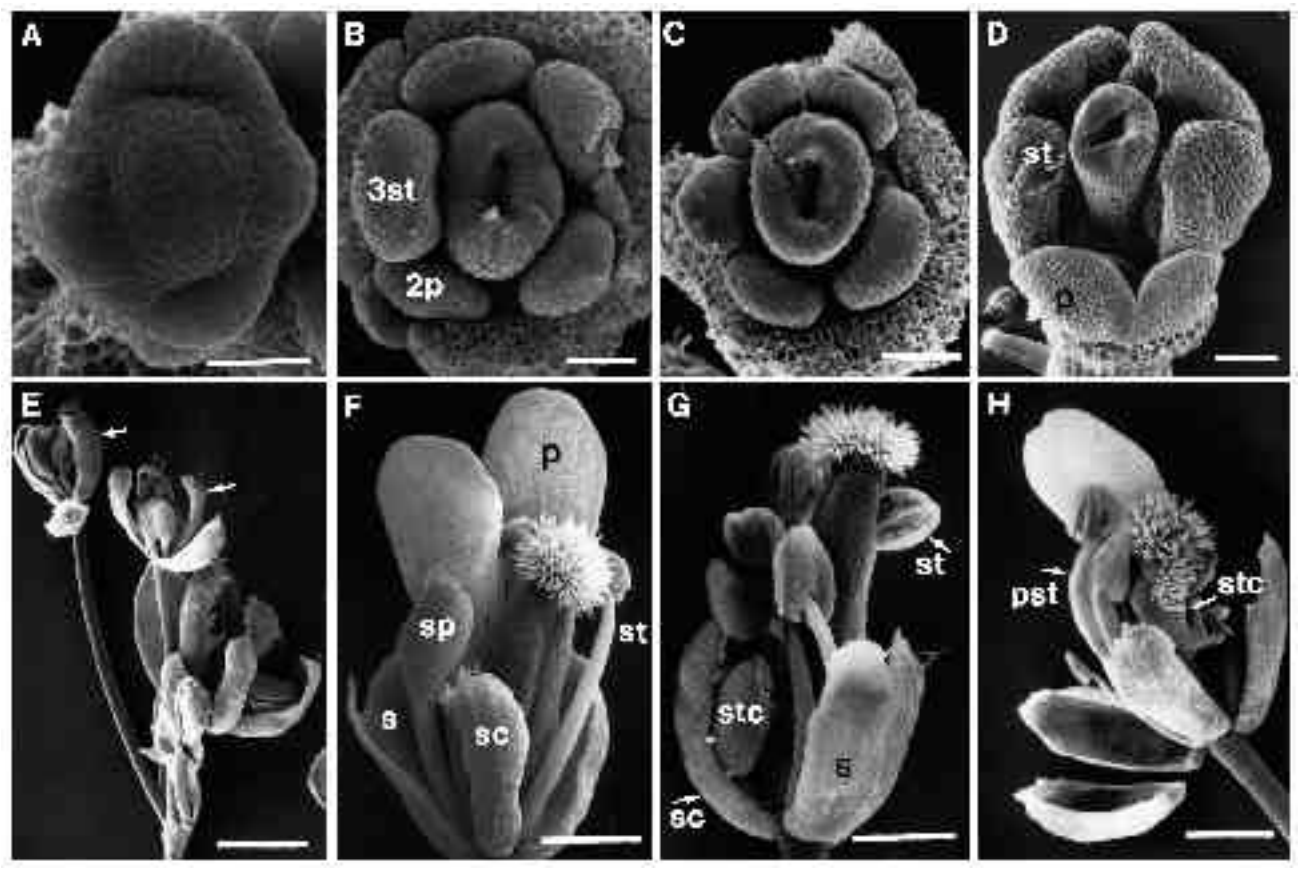


Fig. 5. Scanning electron micrographs illustrating the development of 2° shoots taken from the upper inflorescence of Lfy-2. (A) Four first whorl organs are initiated in a whorled arrangement indistinguishable from wild type. (B) Initiation of second and third whorl organs is abnormal. Note that only one second whorl petal primordia (2p) is visible, and five third whorl organs (3st) are initiated at positions not completely corresponding to wild-type third whorl positions. The development of the gynoecial cylinder appears to be like wild type. (C) Further development of second and third whorl organs. No organs are seen in second whorl positions, and only five organs develop in abnormal third whorl positions. Development of the gynoecial cylinder continues as in wild type. (D) Further development of second and third whorl organs. Two organs with cell types typical of petals are developing (p), and four organs with cell types typical of stamens (st). At this stage, the whorl positions of the organs cannot be determined. (E) Mature coflorescence-like 2°. The 2° shoot produces increasingly carpelloid leafy organs, several of which subtend 3° shoots (arrows). (F) Mature flower-like 2°. The outer whorl consists of four sepals (s) or sepal-carpels (sc), with stigmatic papillae. In second and third whorls, a sepal-petal (sp) as well as petals (p) and stamens (st) are visible. A wild-type gynoecium forms the fourth whorl. (G) Mature flower-like 2°. The outer whorl consists of sepals (s) or sepal-carpels (sc). Second and third whorls consist of stamens (st) and stamen-carpels (stc). A wild-type gynoecium forms the fourth whorl. (H) Mature flower-like 2°. The outer whorl consists of four sepals. Second and third whorls consist of stamens (st) and petal-stamens (pst). In place of a wild-type gynoecium, an incompletely fused pistil-like structure consisting of carpels and stamen-carpels (stc) forms. Bars, 30 μm in A and B; 50 μm in C and D; 500 μm in F-H; 1 mm in E.

The phenotypes caused by the five alleles vary considerably, the least extreme producing a number of wild-type flowers and the most extreme being similar to Ap1-1. We have grouped the alleles into three phenotypic classes, with the strongest phenotype having the most coflorescence-like basal 2° shoots, and the most severe floral organ transformations in upper 2° shoots (Tables 1 and 2): weak (*ap1-13*, *ap1-14*, and *ap1-12*), intermediate (*ap1-11*) and strong (*ap1-1*, *ap1-10*). Sequence analysis of both *ap1-10* and *ap1-11* is consistent with their phenotypic strengths, Ap1-10 being the result of a stop codon, and Ap1-11 being the result of an intron donor site mutation (Martin Yanofsky and Alejandra Mandel, personal communication).

Weak Apetala1 phenotype (Ap1-12, Ap1-13, Ap1-14)

The phenotypes of the three weak *AP1* alleles are very similar, and we will describe only that of Ap1-12 in detail. Within the first whorl of 2° shoots, the trend from more to less coflorescence-like characteristics is apparent as one moves acropetally through the inflorescence. Most commonly, four organs arise in what appears to be a whorled arrangement (Fig. 6A), although in basal-most 2° shoots, first whorl organs in lateral positions may be missing or replaced by filaments or small protrusions of tissue (Fig. 6G). Identity of first whorl organs varies with the position of the 2° shoot in the inflorescence, from leaves, to leaf-sepal intermediate organs, and finally to sepals and sepal-carpels (Table 3). Any of the organ types may be flanked by stipules (Fig. 6D-G), although these become less frequent as one moves acropetally through the inflorescence. The frequency of elongation between lateral and axial organs (Fig. 6G) and formation of 3° shoots in the axils of the organs (Fig. 6D,G) decreases from basal to upper inflorescence. The 3° meristems reiterate the phenotype of the 2° shoots, but are always less coflorescence-like than the 2° shoots from which they develop.

Table 3. Numbers[a] and types of organs observed in Lfy and Ap1 flowers

Phenotype	Lfy-2	Ap1-12	Ap1-13	Ap1-11	Ap1-10
Flowers scored[b]	126	104	105	108	132
Whorl 1					
3° Meristems	0.36	1.01	1.03	1.92	1.46
Leaf/leaf-sepal/sepal[c]	2.69	3.21	3.30	1.71	1.00
Sepal-carpel	0.76	0.12	0.06	0.47	0.39
Filament	0.00	0.04	0.21	1.18	0.76
Organ missing	0.55	0.63	0.44	0.64	1.84
Whorl 2[d]					
Sepal	0.01	0.00	0.00	0.00	0.00
Petal-sepal	0.24	0.07	0.06	0.01	0.02
Petal	0.55	0.78	1.82	0.06	0.01
Whorl 2 or 3[e]					
Sepal-stamen	0.13	0.00	0.00	0.00	0.14
Sepal-petal-/stamen	0.02	0.00	0.00	0.00	0.00
Petal-stamen	0.76	0.32	0.23	0.22	0.41
Stamen	3.04	5.66	5.50	5.51	5.34
Filament	0.17	0.89	0.96	0.44	0.44
Organ missing	4.89	2.28	1.43	3.70	5.62
Whorl 3[f]					
Stamen-carpel	0.18	0.00	0.00	0.05	0.03
Whorl 4					
Carpel	1.98	2.00	2.00	2.05	1.77
Stamen-carpel	0.06	0.00	0.00	0.02	0.18
Stamen	0.00	0.00	0.00	0.00	0.06

[a]All numbers indicate the average number of organs observed per flower
[b]Flowers were taken from throughout the inflorescence
[c]Organ type cannot be distinguished by light microscope
[d]Whorl 2 = any organ which was definitely in second whorl position
[e]Whorl 2 or 3 = any organ whose definite position in second or third whorl could not be determined
[f]Whorl 3 = any organ which was definitely in third whorl position

As in Lfy-2, alteration in organ fate and position, combined with organ fusion, loss and/or gain makes assignment of organs to either whorl 2 or 3 inconclusive (Table 3). In basal-most 2° shoots (Fig. 6G), only stamens are seen at maturity. Second whorl organs are usually initiated normally in less basal 2° shoots, and form petals or filaments (Fig. 6H), or rarely petal-stamens and stamens (Table 3). Organs in the position of lateral stamens are commonly missing (Fig. 6E), and organs in axial positions usually form stamens, although filaments or petal-stamens may occur. In some cases, these intermediate organs may be the result of fusion between an organ of the second whorl and a organ arising in the position of a lateral stamen (Fig. 6F). In the fourth whorl a bicarpellate gynoecium develops in a manner indistinguishable from wild type (Fig. 6D-H).

Intermediate Ap1 phenotype (Ap1-11)

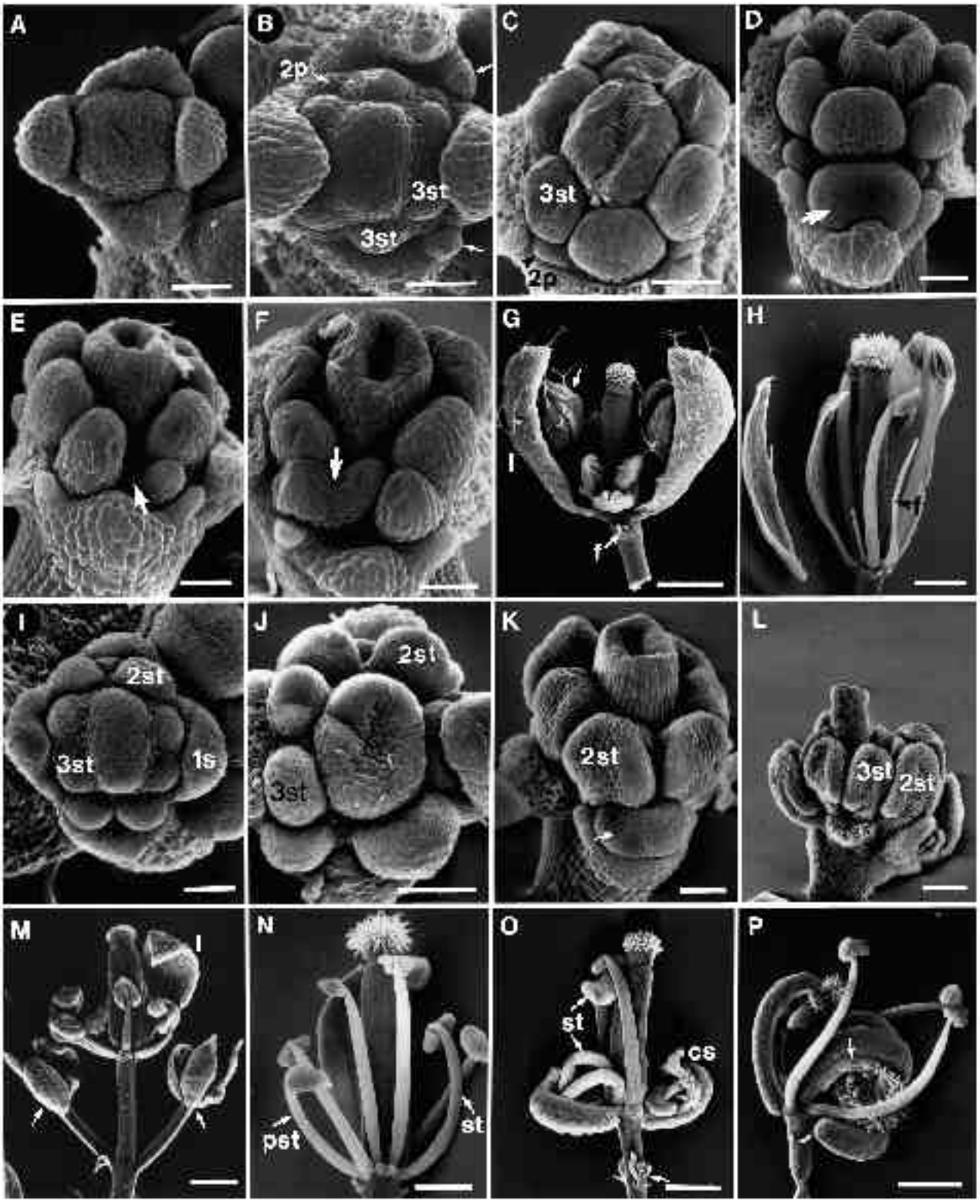
Development of the first whorl of Ap1-11 flowers follows the same trend as is observed in the weak alleles. More commonly than in Ap1-12, organ primordia in lateral positions and occasionally in axial positions form only small protrusions of tissue or small filaments (Fig. 6M-P). More frequently, axial organs develop into leaf-like organs in basal-most 2° shoots (Fig. 6M), or into leaf-carpel or sepal-carpel intermediate organs in upper-most 2° shoots (Fig. 6O,P). These sepal-carpel organs are similar in structure to those seen with strong *AP2* alleles (Kunst et al., 1988), having an central region of sepal tissue, and margins bearing ovule-like structures and stigmatic papillae. Elongation between lateral organs and axial organs (Fig. 6M,O), and development of 3° meristem in axils of lateral organs (Fig. 6M), occur more frequently than in Ap1-12, but show a similar decrease in frequency as one proceeds acropetally through the inflorescence.

Similar organ arrangements and types occur in the second and third whorl organs of Ap1-11 flowers as in Ap1-12, although with greater consistency. In most 2° shoots examined, second whorl primordia appear to be initiated at the correct time and position (Fig. 6I,J), and typically form stamens (Fig. 6J-L,N), although filaments, petal-stamens (Fig. 6N), or petal-sepals may also develop. In the third whorl, the short lateral stamens are often missing (Fig. 6I-K), and the number of axial stamens may be reduced (Fig. 6J). Occasionally, a lateral stamen appears to be transformed into a stamen-carpel intermediate organ. Most commonly in the fourth whorl a normal bicarpellate pistil develops. Occasionally the carpels may be incompletely fused (Fig. 6P), or a three or four carpellate gynoecium may develop. This multicarpellate pistil is always accompanied by lateral stamen loss, suggesting that the extra carpels may be the result of third whorl stamen to carpel transformation. However, many flowers missing lateral stamens have only a bicarpellate

gynoecium, indicating that incorporation into the gynoecium does not account for all absent lateral stamens.

Strong Ap1 phenotype (Ap1-1, Ap1-10)

In the most basal 2° shoots, as many as six nodes bearing 3° meristems, typically with no leafy organ, are initiated in spiral phyllotaxy (Fig. 6Q). 2° shoots taken from the mid-inflorescence initiate organs in an arrangement similar to that seen in basal Ap1-11 2° shoots (Figs 6I,W). In uppermost 2° shoots, lateral organs are missing or form filamentous organs, while axial organs are sepal-carpels (Fig. 6X).



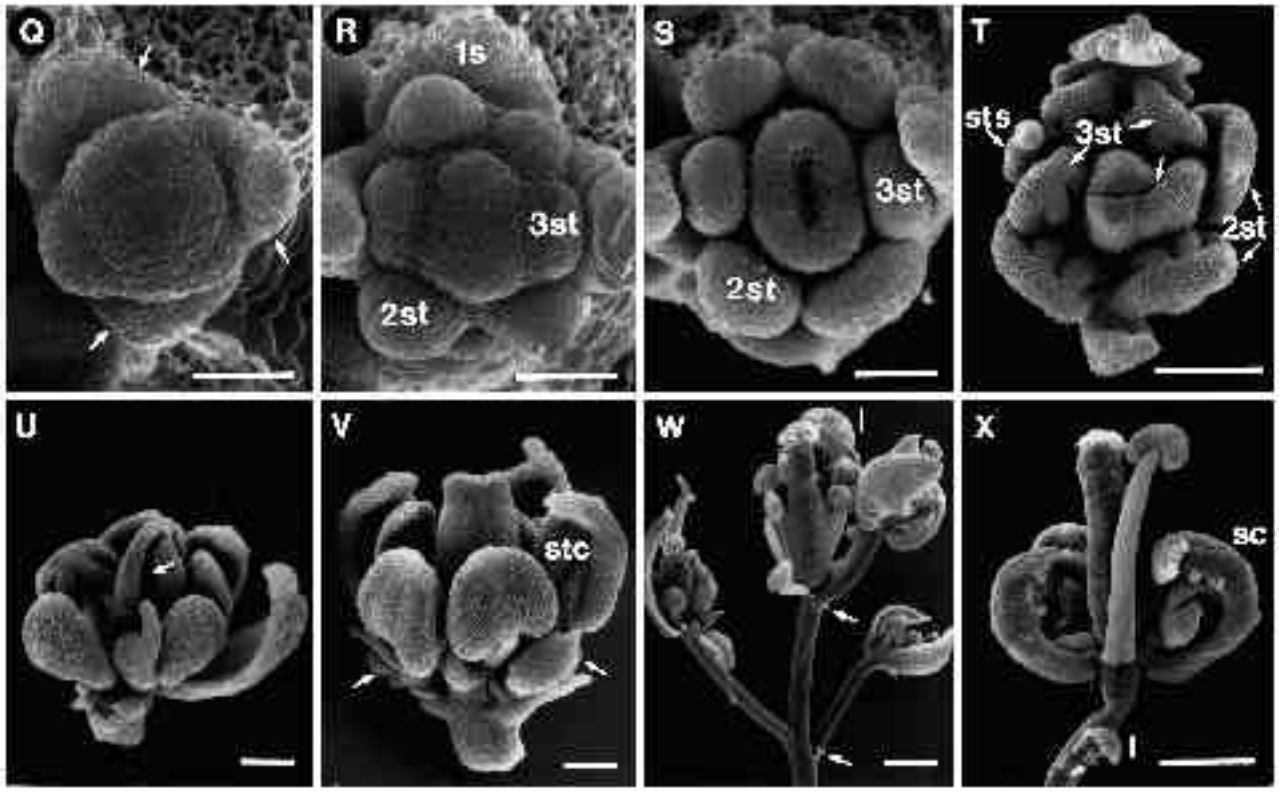


Fig. 6. Scanning electron micrographs illustrating the development of Ap1-12 (A-H), Ap1-11 (I-P) and Ap1-10 (Q-X) 2° flower-like structures. (A) Early stage of Ap1-12 2° meristem development, with four primordia initiated in whorled arrangement. (B) Later stage of Ap1-12 2° meristem development. Organs are initiated in appropriate number and position in first three whorls (2p, 3st). Note stipules developing on the margins of the first whorl organs (arrows). (C) Later stage of Ap1-12 2° meristem development. First whorl organs have been removed. Development of second (2p) and third (3st) organs is indistinguishable from wild type. The gynoecial cylinder is beginning to form. (D) Later stage of Ap1-12 2° meristem development. Axial first whorl organs have been removed. Organs in all four whorls develop normally. Note the presence of a 3° meristem (arrow) in the axil of an axial first whorl organ. (E) Ap1-12 flower from which axial first whorl organs have been removed, and in which one lateral stamen primordium has not developed (arrow). (F) Ap1-12 flower from which axial first whorl organs have been removed, and in which a second whorl primordium appears to be fused to a lateral stamen primordium (arrow). (G) Mature Ap1-12 flower taken from basal inflorescence. Note that lateral first whorl organ is replaced by a filament (f) flanked by stipules, and axial first whorl organs are replaced by leaves (l) which subtend 3° flowers (arrows). (H) Mature Ap1-12 flower taken from upper inflorescence. Note transformation of petals to filaments (f). (I) Early Ap1-11 2° meristem development. Four first whorl organs (1s) are initiated in whorled arrangement, followed by four organs in appropriate second whorl positions (2st). Note that development of second whorl organs is accelerated relative to those in Ap1-12 (Fig. 6B-D). Four organs are initiated in medial third whorl positions (3st), but no primordia form in axial positions. (J) Later Ap1-11 2° meristem development. Second whorl organs (2st) continue to show accelerated development. Only 3 organs develop in third whorl (3st). (K) Later Ap1-11 2° meristem development. Second whorl organs show characteristics of stamens (2st). Note development of 3° meristem (arrow) in axil of axial first whorl organ. (L) Later Ap1-11 2° meristem development. Second whorl organs (2st) appear to be morphologically normal stamens, indistinguishable from third whorl organs (3st). (M) Mature Ap1-11 flower-like 2° from basal inflorescence. Lateral first whorl organs do not develop, but 3° flowers develop in lateral positions (arrows). A leaf (l) has developed in place of an axial first whorl organ. Note elongation between lateral and axial first whorl nodes. (N) Mature Ap1-11 flower from upper inflorescence. Note that second whorl organs are morphologically normal stamens (st), or petal stamen (pst) intermediate organs. Lateral stamens do not develop. (O) Mature Ap1-11 flower from upper inflorescence. First whorl lateral organs are replaced by filaments which subtend 3° meristems (arrow). First whorl axial organs are replaced by carpel-sepal intermediate organs (cs). All second and third whorl organs are stamens (st), and their number is reduced. (P) Mature Ap1-11 flower from upper inflorescence. First three organ whorls similar to Fig. 5O. Fourth whorl is occupied by two incompletely fused carpels (arrow). (Q) Early development of an Ap1-10 2° shoot from basal inflorescence. Note that organ primordia are initiated in spiral arrangement (arrows). (R) Early Ap1-10 flower. First whorl organs (1s) are in whorled arrangement. Organs in second whorl (2st) are initiated in appropriate position, but enlarge more rapidly than would wild-type petals. Only two primordia are initiated in the third whorl (3st). (S) Early Ap1-10 flower. The four organs of second (2st) and two organs of the third whorl (3st) enlarge at a comparable rate. A gynoecial cylinder is developing in a manner similar to wild type. (T) Early Ap1-10 flower. Three stamens (2st) and one stamen-sepal intermediate organ (sts) develop in the second whorl. Three stamens (3st) develop in the third whorl, and in the fourth whorl an abnormal gynoecium develops, to which one third whorl organ appears to fuse (arrow). (U) Early Ap1-10 flower. The gynoecium consists of two incompletely fused organs (arrow). (V) Early Ap1-10 flower. 3° meristems develop in outer whorl positions (arrows). In the third whorl, stamen-carpel intermediate organs (stc) develop. (W) Mature Ap1-10 flower taken from basal inflorescence. Only one first whorl leafy organ develops (l). At the other first whorl positions 3° flowers form, which may have stipules at their base (arrows). (X) Mature Ap1-10 flower taken from upper inflorescence. Lateral first whorl organs are replaced by reduced leaf-like organs (l). Axial first whorl organs are replaced by sepal-carpel intermediate organs (sc), bearing ovule-like structures and stigmatic papillae. Bars, 30 μm in A-F,I-K and Q-S; 100 μm in L and T-V; 500 μm in G,H,M-P,W and X.

Development of the second and third whorls in Ap1-10 2° shoots is similar to that described for Ap1-11. Four primordia, initiated in positions consistent with their representing second whorl organs, develop into stamens, stamenpetals or filaments. In the third whorl, organs in lateral positions are consistently missing, and numbers of axial third whorl organs is also reduced (Fig. 6R). The remaining two to three third whorl organs most frequently develop into stamens. Thus, a set of six stamens (Fig. 6S) may arise from both second and third whorls. Third whorl organs may also form stamen-carpel organs (Fig. 6T,V), which fuse, partially or completely, to the developing gynoecium, resulting in a multicarpellate structure (Fig. 6T).

Finally, in the fourth whorl, a gynoecial cylinder typically forms (Fig. 6S,V-W). Occasionally the carpels may remain unfused (Fig. 6U), or may be replaced by a number of stamens. Often the fertility of these flowers is reduced, with stamens shedding little pollen, and ovules appearing to degenerate.

The Ap1-1 phenotype has been described as a replacement of sepals by a whorl of leaves subtending 3° shoots, and an absence of the petal whorl (Irish and Sussex, 1990). That organs are produced in whorled rather than spiral phyllotaxy suggests that *ap1-1* should be considered a weak *AP1* allele. However, unlike the weak phenotype Ap1-12, Ap1-1 produces neither normal sepals nor petals. Thus, we suggest that the whorl-like arrangement of the organs is the result of the Ler genetic background rather than a weak *AP1* allele, and have classified *ap1-1* as a strong *AP1* allele.

Photoperiod affects the Ap1 phenotype

To determine if, like the *LFY* alleles, the *AP1* alleles are influenced by photoperiod, we grew Ap1-1, Ap1-10, Ap1-13 and Ap1-12 plants under SD conditions. Ap1-1, Ap1-10 and Ap1-13 plants have a similar BT with no fewer rosette nodes than the wild-type ecotype (Table 2). However, Ap1-12 have an earlier BT with fewer rosette nodes than wild type (Table 2). Like wild type, all alleles then produce a number of leaves subtending coflorescences. Following this stage, allelic differences become apparent. In the most extreme case, Ap1-10, all 2° meristems in the inflorescence form coflorescence-like structures (Fig. 3). In Ap1-1 plants, the first 2° shoots not subtended by leaves are transformed into coflorescences. However, following this basal region, 2° shoots are initiated, which are similar to those formed in Ap1-1 under CL. We suggest that the phenotypes of the two strong alleles, *Ap1-1* and *Ap1-10*, differ from one another under SD as a result of differences in Ler and Columbia backgrounds. With weak alleles such as *Ap1-12* the phenotype of the basal 2° shoots are more coflorescence-like than under CL such that they produce 4-8 leaves subtending 3° laterals and separated by internode elongation (Fig. 3). Following this region, six stamens and a bicarpellate pistil form normally. The upper 2° shoots of Ap1-12 plants form flowers similar in structure to those in CL.

Ap2 phenotypes in SD photoperiod

Certain alleles of *AP2* have some characteristics which suggest that, like *AP1, AP2* may have some function in suppressing the coflorescence program. For example, Ap2-1 inflorescences produce 2° shoots in which the sepal whorl is replaced by leafy organs or leaf-carpel organs (Bowman et al., 1991). As well, the phenotypes of certain alleles appear to be affected by photoperiod (Komaki et al., 1988).

We grew plants homozygous for *ap2-1, ap2-5* and *ap2-6* under SD conditions and analyzed the resulting phenotypes. Neither number of rosette nodes nor number of coflorescence nodes were different than in wild-type plants grown under SD (data not shown). Flowers of the weak *Ap2-5* allele were not significantly different from those seen under CL. However, the phenotypes of both Ap2-1 and Ap2-6 were significantly altered. As under CL, the first whorl of Ap2-1 flowers consists of leafy organs, but under SD these frequently subtend 3° shoots (data not shown). Whereas under CL the second whorl of Ap2-1 flowers consists of petal-stamens, under SD, leafy organs arise. Under CL, the first and second whorls of Ap2-6 flowers are transformed to carpels (Kunst et al., 1989); under SD, both whorls are transformed to leafy organs, which often subtend 3° shoots and may be separated by internode elongation (data not shown).

Double mutant analysis

The homeotic transformations of 2° meristems within the inflorescence of Tfl1-14 plants may be described as opposite to those in Lfy and Ap1 inflorescences. The photoperiodic responses of the mutants are also opposite. SD conditions suppress the Tfl1-14 phenotype but enhance the phenotype of Lfy and Ap1. To determine how *TFL1, LFY* and *AP1* interact to regulate the transition from early to late inflorescence, we constructed doubly and triply mutant lines. As well, because homeotic transformations of organs in Lfy and Ap1 2° shoots suggest interactions of these genes with the whorl identity genes, we constructed lines doubly mutant for mutations in either Classes A and C organ identity genes and either *LFY* or *AP1*.

Leafy/Terminal flower 1

Lines doubly mutant for either *lfy-1* or *lfy-2* and *tfl1-14* form an inflorescence after a shorter time but with no fewer rosette nodes than the Tfl1-14 single mutant. Both doubly mutant lines produce fewer coflorescence and leaf bearing nodes than the corresponding Lfy single mutant (Table 2, Fig. 3), followed by nodes bearing flower-like 2° shoots. Thus, the 2° shoots of the double mutants are similar in form to those of the corresponding Lfy single mutant, but occur at an earlier node on the 1° shoot. The total number of inflorescence nodes produced is greater than that in Tfl1-14 single mutants, but significantly fewer than Lfy or wild type (Table 2, Fig. 3). In both doubly mutant lines, 1° and 2° inflorescence shoots end in a number of partially fused carpels or leaf-carpel organs.

Terminal flower 1/Apetala1

In order to determine if loss of *TFL1* activity has any effect on the Ap1 phenotypes, we generated lines doubly mutant for *tfl1-14* and both the strong alleles *ap1-1* and *ap1-10* and the weak alleles *ap1-13* and *ap1-14*. Because of the similar phenotypes between Tfl1-14/Ap1-13 and Tfl1-14/Ap1-14, as well as between Tfl1-14/Ap1-1 and Tfl1-14/Ap1-10, we will describe only Tfl1-14/Ap1-10 and Tfl1-14/Ap1-13.

Plants doubly mutant for *tfl1-14* and either *ap1* allele have the same BT and number of rosette nodes as the Tfl1-14

mutant. They then produce a number of Ap1-like 2° shoots, which become less coflorescence-like as one proceeds acropetally; the first 1-2 are subtended by a leaf, and the remaining 2-3 by no leaf. The phenotypes of the 2° shoots are essentially similar to 2° shoots formed in the corresponding Ap1 plant, but are less coflorescence-like than those formed at a corresponding node in the Ap1 single mutant. In the Tfl1-14/Ap1-13 double mutant, the 1° meristem forms a flower similar in phenotype to an Ap1-13 flower. In contrast, the 1° meristem of Tfl1-14/Ap1-10 double mutants forms a number of incompletely fused carpelloid organs.

Leafy/Apetala1

The *LFY* mutation affects the fate of both the subtending leaf and 2° meristem, while Ap1 affects only the fate of 2° meristems. Moreover, *LFY* mutations result in 2° shoots that are more coflorescence-like than any *AP1* allele. Together, these observations suggest that *LFY* has a more profound role in the switch from coflorescence to floral fates than

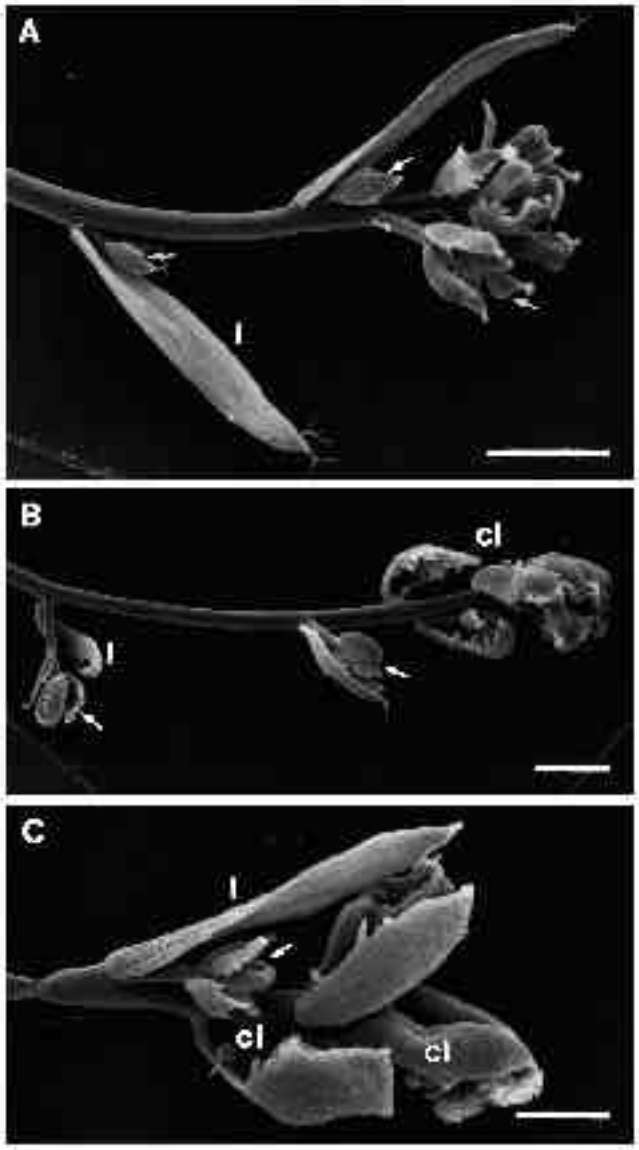


Fig. 7. Scanning electron micrographs of 2° shoots from Lfy-2/Ap1-10 double mutants. (A) Extreme coflorescence-like shoot from basal inflorescence, with leaves (l) subtending 3° shoots (arrows). (B) Coflorescence-like shoot from mid-inflorescence showing gradual transition of organ type from leaves (l) to carpelloid-leaves (cl), which may subtend 3° shoots (arrows). (C) Coflorescence-like shoot from upper inflorescence, with few 3° shoots and little internode elongation between leaves (l) and carpelloid leaves (cl). Bars, 500 μm in C; 1 mm in A and B.

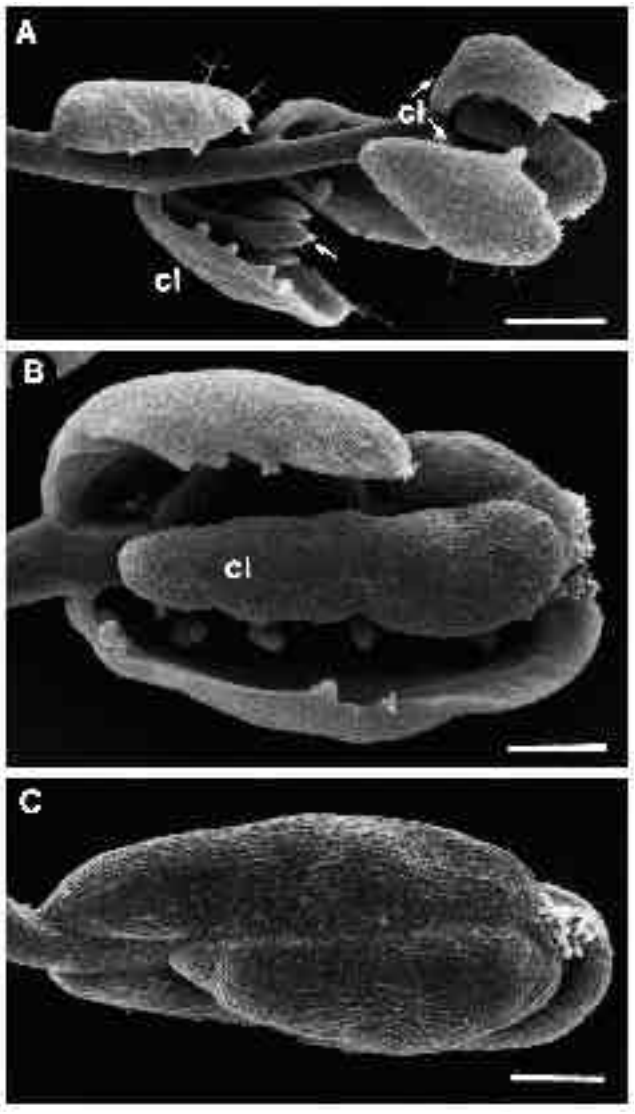


Fig. 8. Scanning electron micrographs of 2° shoots from Lfy-1/Ap1-1/Tfl1-14 triple mutants. (A) Coflorescence-like shoot from basal inflorescence, with carpelloid-leaves (cl) subtending 3° meristems (arrows) and separated by internode elongation. (B) Flower-like shoot from mid-inflorescence, with carpelloid-leaves (cl) separated by no internode elongation and fusing to form a pistil-like structure. (C) Flower-like shoot from upper inflorescence, consisting only of carpels and carpelloid leaves fused to form a pistil-like structure. Bars, 300 μm in B and C; 500 μm in A.

AP1. Previous double mutant constructions suggest that loss of *AP1* results in a more extreme Lfy phenotype (Huala and Sussex, 1992; Weigel et al., 1992). To determine if allelic differences would influence the double mutant phenotypes, we constructed lines doubly mutant for strong and weak alleles of both *LFY* and *AP1*, generating the following lines: Lfy-1/Ap1-1; Lfy-1/Ap1-10; Lfy-2/Ap1-1; Lfy-2/Ap1-10; Lfy-2/Ap1-12.

Rather surprisingly, the double mutant phenotypes of all allelic combinations are similar, resulting in inflorescences consisting only of coflorescence-like structures. Because all allelic combinations give similar phenotypes, we will describe only Lfy-2/Ap1-10. Doubly mutant inflorescences produce the same number of nodes with leaves and coflorescences as Lfy-2. However, no flower-like structures are ever produced in the doubly mutant lines, although as in the single mutants, there is an acropetal decrease in coflorescence-like characteristics (compare Fig. 7A with 7C). All 2° shoots appear to end in a malformed pistil-like structure (Fig. 7B,C) similar to those seen in Lfy mutants.

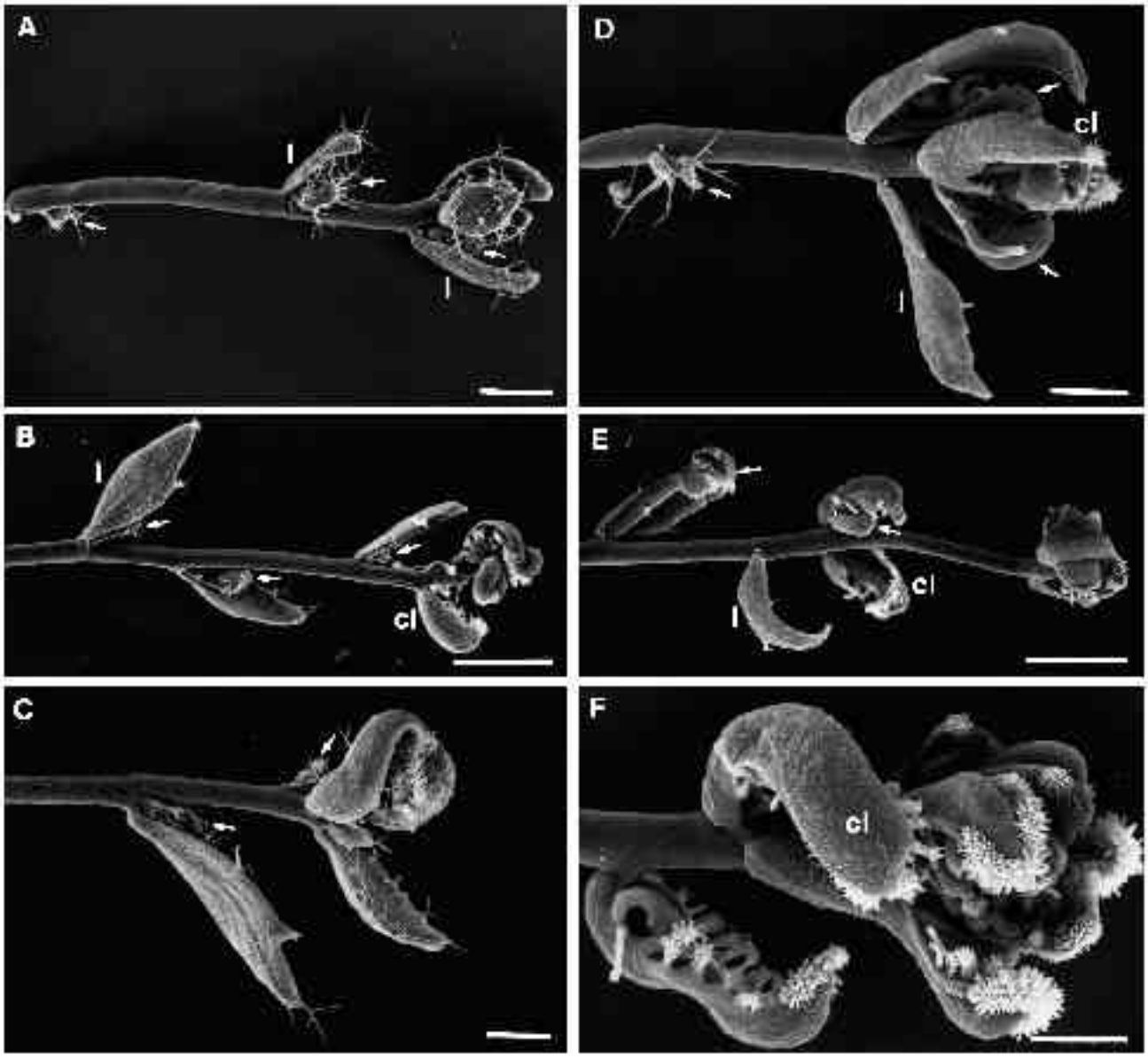


Fig. 9. Scanning electron micrographs of 2° shoots from Lfy-1/Ap2-6 (A-C) and Lfy-2/Ap2-6 (D-F) double mutants. (A) Extreme coflorescence-like shoot from basal inflorescence, with leaves (l) subtending 3° shoots (arrows). (B) Coflorescence-like shoot from mid-inflorescence showing gradual transition of organ type from leaves (l) to carpelloid-leaves (cl) which may subtend 3° shoots (arrows). (C) Coflorescence-like shoot from upper inflorescence, with little internode elongation between organs and few 3° shoots (arrows). (D) Coflorescence-like shoot from basal inflorescence, with leaves (l) and carpelloid leaves (cl) subtending 3° shoots (arrows). (E) Coflorescence-like shoot from mid-inflorescence, showing gradual transition of organ type from leaves (l) to carpelloid-leaves (cl), which may subtend 3° shoots (arrows). (F) Coflorescence-like shoot from upper inflorescence, in which all organs are carpelloid leaves (cl). No 3° shoots develop, and there is little elongation between organs. Bars, 500 μm in A,C,D and F; 1 mm in B and E.

Leafy/Terminal Flower 1/Apetala1

Plants triply mutant for *TFL1*, *LFY* and *AP1* form an inflorescence at the same time as Tfl1-14 mutants, and with the same number of rosette nodes. They then produce the same number of leaves as does the Tfl1-14/Lfy-1 double mutant, before the 1° meristem forms a pistil-like structure similar to that in the Tfl1-14/Lfy-1 double mutant. In the axils of these leaves, 2° shoots develop (Fig. 8), which are similar in form to the 2° shoots of a Lfy-1/Ap1-1 double mutant. Basal 2° shoots are coflorescence-like (Fig. 8A), but 2° shoots become rapidly less coflorescence-like as one moves acropetally through the inflorescence (Fig. 8B), such that uppermost 2° shoots are more flower-like than those at a corresponding node in the Lfy-1/Ap1-1 double mutant, consisting only of fused leaf-carpel organs (Fig. 8C).

Leafy/Apetala2

We have generated lines doubly mutant for *lfy-1* or *lfy-2* with *ap2-1* and *ap2-6*. The phenotype of Lfy-1/Ap2-1 and Lfy-2/Ap2-1 are more similar to lines doubly mutant for *LFY* and *AP1* than those double mutant combinations described by Huala and Sussex (1992). Inflorescences fail to produce flower-like 2° shoots, but rather continue to form 2° shoots having leaves and leaf-carpel intermediate organs subtending 3° meristems and separated by elongated internodes (data not shown). In contrast to lines constructed by

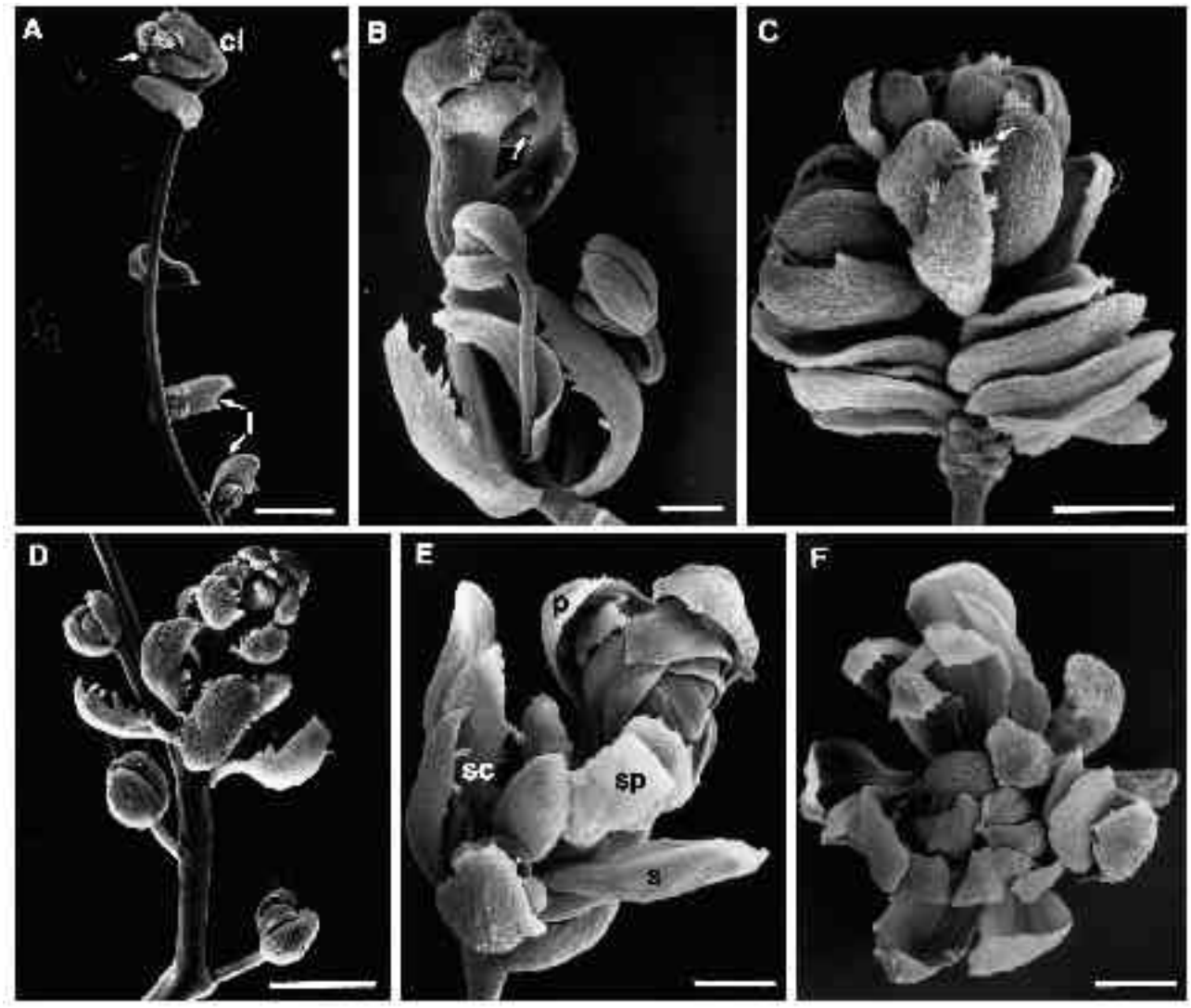


Fig. 10. Scanning electron micrographs of 2° shoots from Lfy-1/Ag (A-C) and Lfy-2/Ag (D-F). (A) Lfy-1/Ag coflorescence-like shoot from basal inflorescence showing gradual transition of leaves (l) to carpelloid-leaves (cl), and incomplete fusion of carpelloid-leaves into a pistil-like structure (arrow). (B) Lfy-1/Ag coflorescence-like shoot from mid-inflorescence, in which organs are leaves or sepals, all with some carpelloid characteristics such as stigmatic papillae and ovule-like projections (arrow). (C) Lfy-1/Ag flower-like shoot from upper inflorescence, in which all organs are sepals, some with carpelloid characteristics such as stigmatic papillae (ovule). (D) Lfy-2/Ag coflorescence-like shoot from basal inflorescence. All organs are carpelloid leaves. The fusion between organs is incomplete, such that a pistil-like structure never forms. (E) Lfy-2/Ag flower-like shoot from mid-inflorescence. Organs consist of sepals (s), petals (p), sepal-petals (sp) and sepal-carpels (sc). Note the formation of sepals having carpelloid characteristics in the outermost whorl. (F) Lfy-2/Ag flower-like shoot from upper inflorescence. Organ types as in E. Bars, 500 μm in B,C,E and F; 1 mm in A and D.

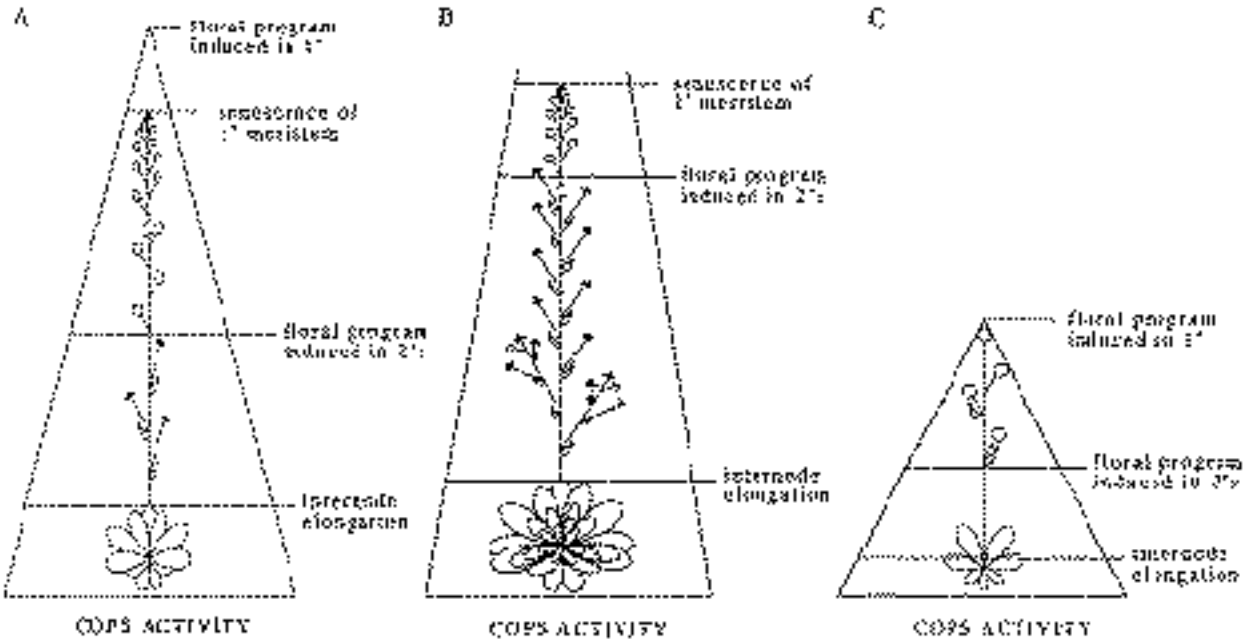


Fig. 11. Diagrammatic representation of a mechanism controlling phase switching (COPS) in (A) wild type grown under CL, (B) wild type grown under SD conditions and (C) Tfl1 grown under continuous light. Symbols as in Fig. 1. The gradual change in the activity of the COPS factor(s) throughout plant development is indicated by the changing width of the triangle. Phase switching is initiated at critical levels of COPS activity, as shown by the horizontal lines crossing the triangle. Genes such as *TFL1*, *EMF*, and the late-flowering loci, as well as environmental factors such as photoperiod, influence the rate of decrease (compare A with B and C), and thus the time of phase-switching. At the COPS activity level, which results in the early to late inflorescence phase switch, the FLIP genes (*LFY*, *AP1* and *AP2*) are activated in nodal primordia (see Fig. 12). Analysis of Tfl1 inflorescences suggests that if COPS activity decreases sufficiently (the apex of the triangle), the FLIP genes can be induced in the 1° meristem. However, in wild-type plants, the 1° meristem senesces before COPS levels become low enough (compare Fig. A with C).

Huala and Sussex (1992), the 2° shoots of either Lfy-1/Ap2-1 or Lfy-2/Ap2-1 end in a number of partially fused leaf-carpel intermediate organs.

We have presented a brief description of Lfy-1/Ap2-6 double mutants previously (Schultz and Haughn, 1991). Doubly mutant plants produce flower-like 2° shoots much more rarely than Lfy-1 inflorescences. Instead, the inflorescence consists almost entirely of coflorescence-like shoots similar to those in Lfy-1 single mutants, having organ types ranging from leaves, through sepals and carpels (Fig. 9A-C). Lines doubly mutant for *lfy-2* and *ap2-6* do eventually form flower-like 2° shoots (Fig. 9F), although at a later node than either Ap2-6 or Lfy-2 single mutant inflorescences. The coflorescence-like 2° shoots (Fig. 9D,E) are similar to those formed in Lfy-1/Ap2-6 doubles, while the more flower-like uppermost 2° shoots are composed entirely of carpels (Fig. 9F).

Leafy/Agamous

Like *AP2*, both *LFY* (Huala and Sussex, 1992) and *AP1* (Mandel et al., 1992) also appear to influence Class C gene expression, since in Lfy-1, Lfy-2, Lfy/Ap1 and Lfy/Ap2-1 2° shoots, increasingly carpel-like organs are initiated (leaf, leaf-carpel, carpel). Moreover, analysis of Lfy/Ap2-6 double mutants suggests that absence of *AP2* does not greatly alter the pattern of Class C gene expression in Lfy coflorescence-like 2° shoots. In order to determine if the carpel-like organs that form in Lfy coflorescence-like 2° shoots require *AG*, we generated lines doubly mutant for *lfy-1* or *lfy-2* and *ag-1*. Doubly mutant lines with *ag* and other *LFY* alleles have been described previously (Huala and Sussex, 1992; Weigel et al., 1992).

Organ types within Lfy-1/Ag-1 and Lfy-2/Ag-1 coflorescence-like 2° shoots do not show a dramatic alteration from those in the Lfy single mutant plants, having similar organ types, and ending in partially fused carpels or leaf-carpels (Fig. 10E). The degree of carpelloidy appeared to be somewhat reduced in these structures, especially in Lfy-2/Ag-1, such that fusion was rarely as complete as in the Lfy single mutants.

Double mutant 2° shoots formed at a node corresponding to the most flower-like 2° shoots in the Lfy single mutant are similar to those described by Huala and Sussex (1992). Lfy-1/Ag-1 flower-like 2° shoots consist of sepals and sepal-carpel intermediate organs, and Lfy-2/Ag-1 of sepals, rare sepal-carpel intermediate organs and petals or petal-sepal intermediate organs. In both Lfy-1/Ag-1 (Fig. 10B) and Lfy-2/Ag-1 (Fig. 10E), organs frequently subtend 3° meristems. Moreover, although internodes between the floral organs are compressed early in development, as the plant matures, the internodes elongate. Thus, loss of *AG* appears to increase coflorescence-like characteristics as well as decreasing numbers of reproductive organs.

Apetala1/Apetala2

We generated double mutants between *ap1-1* and each of

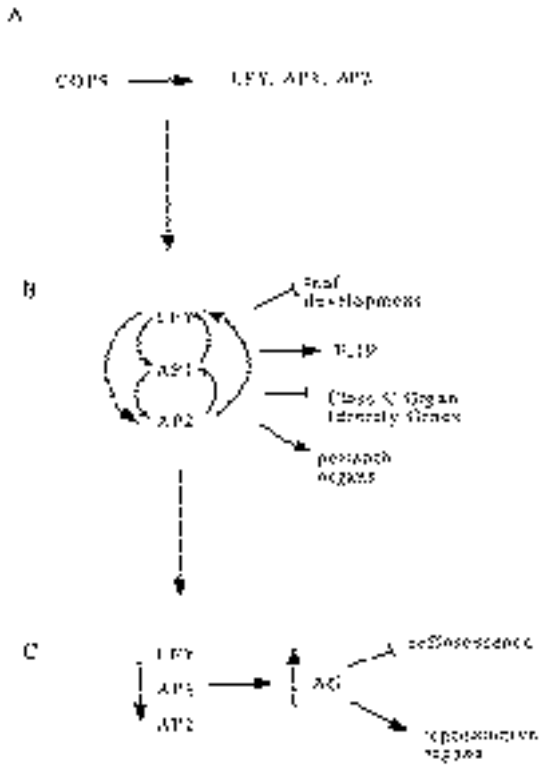


Fig. 12. Proposed sequence of gene expression during development of nodes in the late inflorescence phase. j = promotes; ¾½= inhibits. (A) Very early development. FLIP genes are activated independently by COPS, *LFY* in both 2° meristem and subtending organ primordia, *AP1* and *AP2* only in 2° meristem. (B) Early development. *LFY* suppresses development of the subtending leaf. *LFY*, *AP1,* and *AP2* act cooperatively to suppress the coflorescence program and activate the floral initiation program (FLIP) in the 2° meristem. As well, the genes restrict Class C organ identity gene expression from the perianth, allowing development of perianth organs. (C) Late development. Expression of the FLIP genes decreases, allowing expression of Class C genes and development of reproductive organs. In addition, Class C gene expression is required for maintenance of the floral program through repression of the coflorescence program.

ap2-1, *ap2-5* and *ap2-6*. As reported by Irish and Sussex (1990), plants doubly homozygous for *ap1-1* and *ap2-1* were found to produce very coflorescence-like 2° shoots in place of flowers (data not shown). These are especially coflorescence-like at the base of the inflorescence, and become less so as one proceeds acropetally. Such coflorescence-like 2° shoots were also seen in the basal-most 2° shoots of Ap1-1/Ap2-6 double mutants, although at a low frequency (data not shown). Except for these basal-most 2° shoots, the phenotypes of the outer whorls of Ap1-1/Ap2-6 and Ap1-1/Ap2-5 were essentially additive of the single mutant phenotypes, while the phenotype of the inner whorls was more extreme than either single mutant. 2-4 3° shoots form first in Ap1-1/Ap2-6 2° shoots, and may be subtended by no organ or by leaf-carpel organs, and be separated by internode elongation (data not shown). Next, several stamens, stamen-carpel intermediate organs and finally carpels develop. In some flowers, two carpels fuse to form a typical bicarpellate pistil. However, more frequently than in either single mutant, carpels appear abnormal in morphology and remained unfused.

DISCUSSION

During development of the *Arabidopsis* shoot, the primary apical meristem switches from producing leaf- and coflorescence-bearing nodes to producing flower-bearing nodes. Three genes, *TFL1*, *LFY* and *AP1* are known to affect this process (Irish and Sussex, 1990; Schultz and Haughn, 1991; Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992; Huala and Sussex, 1992; Mandel et al., 1992; Weigel et al., 1992; Shannon and Meeks-Wagner, 1993). In this manuscript we have (1) described four new mutant alleles of *TFL1*, one new allele of *LFY*, and five new alleles of *AP1* genes and (2) analyzed interactions between *TFL1*, *LFY*, *AP1*, *AP2* and *AG* by constructing double and triple mutants. On the basis of these studies we propose a working model for the control of the transition from coflorescence-bearing to flower-bearing nodes.

The control of phase switching in the *Arabidopsis* shoot

The transition from vegetative to reproductive phases of *Arabidopsis* shoot development is facultative and involves changes in leaf shape, internode elongation and lateral shoot type. The most dramatic morphological change occurs when the primary meristem switches from producing a leaf- and coflorescence-bearing node to a flower-bearing node. The number of leaf-bearing nodes that develop prior to the formation of the first flower is strongly regulated by environmental conditions such as photoperiod and temperature (recent review Martinez et al., 1993). As has been suggested for other species (Lang, 1965; Murfet, 1989; Poethig, 1990; McDaniel et al., 1992) *Arabidopsis* must have a mechanism that monitors developmental time and activates morphological programs associated with phase transition in response to the number of nodes produced. In addition, the mechanism must be responsive to the environment (a broader discussion will be given in a subsequent publication: Haughn et al., unpublished data). A simple working model consistent with all the above observations is graphically illustrated in Fig. 11. We suggest that the activity level of a factor(s) changes with node production, and that at critical levels, morphological programs associated with a phase change are activated. We will refer to the factor(s) as **C**ontroller(s) **o**f **P**hase **S**witching (COPS). Moreover, variations in environmental conditions such as photoperiod must accelerate or decelerate COPS change (compare Fig. 11A with B), resulting in a concomitant decrease or increase, respectively, in the time to phase transitions. For simplicity throughout this discussion, we present the change in COPS activity as a decrease (Fig. 11), a hypothesis favoured by the phenotype of the early flowering mutant Embryonic Flower (EMF) (Sung et al., 1992) (see following section). Support for the concept that COPS change is gradual, occurs throughout shoot development and is lower in the lateral

shoot than in the shoot from which the lateral arose, is discussed in following sections.

Fate of apical meristems distinct from lateral meristems

An interesting aspect of plant development is that at any point in development the fate of the apical meristem may be distinct from that of the lateral meristems it generates (Poethig, 1990; McDaniel et al., 1992). For example, in the late inflorescence phase of *Arabidopsis*, lateral meristems follow the floral program, while the 1° meristem pursues an inflorescence program. This reduction in inflorescence-like characteristics in lateral as compared to apical meristems is reiterated in all mutants we have analyzed. In Tfl1 inflorescences, 2° meristems adopt a floral fate several nodes before the 1° meristem is converted to the floral program. In Lfy and Ap1 inflorescences, 3° shoots are always more flower-like than the 2° shoot from which they are derived.

We have suggested that the switching between phases is the result of changing COPS activity. The observations described above suggest that phase changing is accelerated in lateral meristems relative to the apical meristems. This suggests that COPS activity is reduced in lateral meristems relative to the apical meristem from which it originated.

TFL1 affects the activity of COPS

The Tfl1 shoot has a reduced number of nodes in rosette, early inflorescence and late inflorescence developmental phases. At the same time, there seems to be no absolute requirement for *TFL1* activity during any developmental phase, since in various double mutant combinations and environmental conditions, all shoot developmental phases can be observed (coflorescence-bearing nodes in Tfl1-14/Lfy double mutants or in Tfl1-14 under SD conditions). Moreover, developmental phases are expressed in the correct sequence, although at an altered rate. These data suggest that the *TFL1* gene product is involved in establishing the timing of phase transition during shoot development, and thus influences COPS activity (Fig. 11C). All Tfl1 mutants are responsive to environmental conditions. Assuming that at least some of the *TFL1* alleles are null or close to null alleles, this indicates that *TFL1* is not required for the photoperiodic response of COPS.

The development of a terminal flower on the Tfl1 1° shoot demonstrates that the primary meristem can itself enter the floral program although it does not do so in the wild-type plant. We suggest that the wild-type plant normally senesces before COPS activity is low enough to induce the floral program in the primary meristem (Fig. 11).

The phenotypes of the early- and late-flowering loci suggest that, like *TFL1*, they influence COPS activity. Mutations in late-flowering loci (for example *FCA*, *FY*, *FD*, *GI*) result in increased numbers of leaf-bearing nodes relative to wild type (Martinez-Zapater and Somerville, 1990; Koornneef et al., 1991), indicating that the wild-type gene products accelerate COPS activity change (Fig. 11B). Mutations in *EARLY-FLOWERING* (*ELF1*, *ELF2*) loci (Zagotta et al., 1992) and in *EMF* (Sung et al., 1992), result in a decreased number of leaf-bearing nodes, implying that they retard COPS change (Fig. 11C). Mutations in *EMF* result in the most dramatic phenotype, such that a determinate inflorescence forms immediately following germination. In addition, the EMF gene is required for photoperiodic and vernalization response of flowering. Such a phenotype suggests that *EMF* may be central to the COPS mechanism.

LEAFY, *APETALA1* and *APETALA2* determine meristem fate in the inflorescence

The available evidence suggests that *LFY*, *AP1* and *AP2* gene products all have a role in switching meristems from an inflorescence program to a floral program (suppressing inflorescence program, activating floral program), a process we will refer to as FLIP (**Fl**oral **I**nitiation **P**rocess). It is also clear that the contribution of the three genes to FLIP is not equal.

The requirement for *LFY* in FLIP is well documented (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992; Shannon and Meeks-Wagner, 1993). In addition, we demonstrate here that *LFY* is required for the FLIP that occurs in the 1° meristem of *Tfl1* inflorescences. However, even in plants homozygous for the *lfy-1* allele, which DNA sequence analysis suggests is a null allele (Weigel et al., 1992), flower-like 2° shoots form in the upper inflorescence. This gradual replacement of coflorescences by flowers in Lfy-1 inflorescences suggests that the FLIP can occur even in the absence of *LFY* activity.

Observations made previously have suggested that besides *LFY*, at least two other genes, *AP1* and *AP2*, are involved in FLIP (Komaki et al., 1988; Irish and Sussex, 1991; Huala and Sussex, 1992; Weigel et al., 1992; Shannon and Meeks-Wagner, 1993). Our analysis has confirmed and extended these observations. 2° shoots in both Ap1 and Ap2 inflorescences have many coflorescence-like characteristics which, as in Lfy, vary both in an inflorescence- and photoperiodic-dependent manner.

Analyses of Lfy/Ap1, Lfy/Ap2 and Ap1/Ap2 doubly mutant lines support the hypothesis that the three genes have redundant roles in FLIP. Absence of either *AP1* or *AP2* in a Lfy background results in an increased tendency towards coflorescence-like 2° shoots, suggesting that in Lfy plants, the eventual formation of flowers in the upper inflorescence is dependent at least in part on *AP1*/*AP2* expression. Ap1/Ap2 double mutants form very coflorescence-like structures in the basal-most 2° shoots (Irish and Sussex, 1990; this study), suggesting that *LFY* activity alone is insufficient to completely initiate FLIP at the appropriate time. Similar types of functional redundancy have been well-documented in animal development (Tautz, 1991).

Because of their common role in determining 2° meristem fate, we designate *LFY*, *AP1* and *AP2* as FLIP genes. The single mutant phenotypes suggest that while all FLIP genes are necessary to suppress the coflorescence and activate the floral program in meristems, their roles are not equivalent. The transformation of flowers to coflorescences is more complete in Lfy inflorescences than in either Ap1 or Ap2 inflorescences. Similarly, Ap1 2° shoots are more coflorescence-like than those of Ap2 inflorescences. Based on these observations, we suggest that the requirement of the genes for FLIP is *LFY* > *AP1* > *AP2*.

It is notable that *ap2-1*, which is known to have reduced ectopic *AG* expression compared to other *AP2* alleles

(Drews et al., 1991), has a more coflorescence-like phenotype in both singly and doubly mutant combinations. As well, more extreme coflorescence-like structures form in Lfy/Ag double mutants compared to Lfy single mutants. These observations suggest that *AG* activity has a role in suppressing coflorescence characteristics.

As well as activating FLIP in 2° meristems, *LFY* acts in another aspect of the early to late inflorescence phase change, affecting the fate of the subtending leaf. Both leaf and 2° meristem are thought to be derived from a common anlage (Weigel et al., 1992), and, in wild type, have tightly coupled fates (coflorescences subtended by a leaf, flowers subtended by no leaf). However, in both Lfy and Tfl1 inflorescences, their fates become uncoupled, such that flowers may be subtended by a leaf or coflorescences subtended by no leaf. This suggests that although the structures may arise from a common anlage, their fates become uncoupled very early in development.

COPS determines time of expression of FLIP genes

Successive 2° shoots in inflorescences of Lfy, Ap1 and Ap2 single and double mutant combinations become gradually less coflorescence-like as one moves acropetally through the inflorescence. Thus, activation of the FLIP genes is coordinate, independent of one another and progressively stronger as inflorescence development proceeds. Moreover, the rate at which FLIP activation occurs is dependent on *TFL1* activity and daylength. Taken together, these data suggest that COPS coordinately activates the FLIP genes, resulting in the morphological changes associated with the late to early inflorescence phase change (Fig. 11).

The phenotype of 1° structures in Tfl1, Lfy, Ap1-10 and doubly mutant combinations suggests that COPS determines the timing of FLIP gene expression in 1° as well as 2° meristems. The phenotype of the Tfl1 terminal flower is similar to Lfy flower-like 2° shoots, suggesting that *LFY* expression in the terminal flower may be reduced compared to 2° flowers. In Lfy/Tfl1-14 and Ap1-10/Tfl1-14 the main inflorescence forms a pistil-like structure, indicating that ectopic *LFY* and *AP1* expression are required for the Tfl1 terminal flower. Thus, in wild-type plants, COPS may maintain an inflorescence program in the 1° meristem by ensuring that the FLIP genes are not expressed prior to senescence.

FLIP genes function cooperatively to control the sharpness of the FLIP transition

As discussed above, the COPS mechanism determines the time of FLIP gene activation, resulting in the coflorescence to floral transition in meristems. In wild-type or Tfl1 plants, the transition is abrupt. In contrast, loss of function of any one of the FLIP genes results in a COPS-dependent, gradual transition from basal coflorescence-like shoots to apical flower-like shoots (Fig. 3). Thus, combined expression of all FLIP genes is required for an abrupt FLIP, indicating that the FLIP genes must in some way act cooperatively. Following initial coordinate activation by COPS, several possible models for such cooperative action are consistent with the data: (1) the FLIP genes up-regulate one anothers expression; (2) the FLIP genes negatively regulate an inhibitory function, such as COPS; (3) the FLIP gene products act in a cumulative fashion to activate downstream genes.

AP1 is a Class A organ identity gene

The requirement for *AP1* in perianth organ identity is well established (Irish and Sussex, 1990; Mandel et al., 1992). Our analysis of several new *AP1* alleles suggests that, like *AP2, AP1* may be considered a Class A organ identity gene.

The flower-like 2° shoots formed in the upper inflorescence of all *AP1* alleles show transformations of perianth organs to reproductive organs. In stronger alleles, third whorl organ number is reduced and gynoecial abnormalities often occur. Such organ transformations are similar to those observed in flowers mutant for *AP2*. The organ transformations suggest that like *AP2, AP1* has some role in restricting Class C gene expression to the inner whorls, and that in its absence, Class C genes are ectopically expressed in the perianth. Molecular analyses (Mandel et al., 1992) indicate that *AP1* is strongly expressed in all whorls of Ag flowers, suggesting that *AG* restricts *AP1* expression to the perianth. Thus, *AP1* seems to share both aspects of *AP2-AG* regulation, being negatively regulated by *AG*, and serving to negatively regulate *AG*. For these reasons, we believe *AP1* should be considered a Class A whorl identity gene.

We suggest that *AP1* and *AP2* gene products act together as Class A genes specifying organ identity. An alternative possibility for the similarities in Ap1 and Ap2 phenotypes is that one gene is necessary for activation of the other. However, Ap2/Ap1 double mutants have a phenotype distinct from either single mutant suggesting that each gene is expressed in the absence of the other (Irish and Sussex, 1990; this study); in support of this, molecular data suggest that young Ap2 floral primordia express *AP1* (Mandel et al., 1992).

A Class A gene equivalent has not yet been isolated in *Antirrhinum majus*. Interestingly, the sequence of *SQUAMOSA* (Huijser et al., 1992) bears significant sequence identity to *AP1* (Mandel et al., 1992). An attractive possibility is that *SQUAMOSA* represents Class A gene activity in *Antirrhinum*.

LFY influences floral whorl identity

Transformations of sepals to carpels, petals to stamens, and stamens to sepals in Lfy flower-like 2° shoots suggest an altered pattern of Class C gene expression (Huala and Sussex, 1992; Weigel et al., 1992). Though similar to those associated with loss of Class A gene expression, the organ transformations are not identical. Thus, while *LFY* seems to have properties of a Class A gene, its role is slightly different from *AP1* and *AP2*. The apparent Class A *LFY* activity may be a reflection of cooperative function among *LFY, AP1*, and *AP2*. In support of this hypothesis, organ transformations become less extreme in upper 2° shoots of weak Lfy alleles, suggesting that the transformations in lower 2° shoots may be due in part to reduced *AP1*/*AP2* expression.

Transformations of petals to sepals and stamens to carpels in Lfy single mutants indicate reduction in *PI/AP3* expression, a hypothesis supported by double mutant analysis (Huala and Sussex, 1992; Weigel et al., 1992). As in Ap2 and Ap1 phenotypes, the reduced Class B gene

expression may be the result of ectopic Class C gene expression. The rare occurrence of stamen-carpels in the fourth whorl suggests that Class B organ identity genes are ectopically expressed in the fourth whorl. Thus *LFY* may have some role in restricting *PI/AP3* expression, perhaps through activation of the *FLO10* gene (Schultz et al., 1991; Bowman et al., 1992).

LFY acts to suppress class C gene functions in inflorescence shoots

The analysis of Huala and Sussex (1992), as well as our own, suggests that *LFY* has some function in the apical meristems of both coflorescences and the main inflorescence. In wild-type plants, both coflorescences and the main inflorescence are indeterminate, never forming differentiated organ structures. In contrast, the inflorescence-like shoots of Lfy mutants (including the 1°) always terminate in pistil-like structures consisting of fused carpels and carpel-leaves. Such structures form in Lfy/Ap2, Lfy/Ap1, Lfy/Tfl1-14, and, to a lesser extent, in Lfy/Ag double mutants. Their development suggests that *LFY* is required to repress Class C organ-identity gene activation late in inflorescence development. Since carpels develop to a certain extent even in Lfy/Ag double mutants, Class C gene activities besides *AG* must be involved.

The fact that the *LFY* gene product not only activates the floral program early in floral meristem development but also represses class C organ-identity gene expression late in indeterminate inflorescence development seems paradoxical. These data could be explained if (1) COPS weakly activates floral specific genes late in inflorescence meristem development (2) low levels of *LFY* gene product repress Class C organ-identity gene expression but are insufficient for floral activation. Through most of plant development, COPS activity remains too high in the apical meristems to allow FLIP gene expression. Late in plant development, COPS activity is reduced sufficiently to allow low level expression of the floral genes including *LFY* and Class C organ-identity genes. The expression of *LFY* is not sufficient to activate the floral pathway, but is sufficient to inhibit Class C gene expression. This hypothesis predicts that *LFY* be expressed late in the development of inflorescence meristems. Weigel et al. (1992) did not detect *LFY* transcript in inflorescence meristems; however, it is not clear if inflorescence meristems very late in development were examined.

Conclusions

Along with Fig. 12, the following points summarize our conclusions regarding the control of the early to late inflorescence phase switch.

1. COPS activity decreases gradually throughout shoot development (Fig. 11).

2. At a critical COPS level, the FLIP genes are activated in nodal anlage that will give rise to 2° shoots (all FLIP genes activated) and leaves (only *LFY* activated).

3. The FLIP genes together initiate FLIP by repressing the inflorescence program, repressing Class C organ identity gene expression and activating perianth development. In addition, *LFY* represses leaf development.

4. Late in flower development, a decrease in FLIP gene expression allows the expression of Class C organ identity genes which, as well as activating reproductive organ development, continues maintenance of the floral program by repressing the inflorescence program.

Note added in proof

While this manuscript was being reviewed, an analysis of genetic interactions between *TFL1*, *LFY*, *AP1*, *AP2* and *CLV1* by Shannon and Meeks-Wagner (1993) was published. Despite the fact that these authors used different alleles than ourselves for many of the genes, we were gratified to find that, in those areas where we overlapped, our data and overall conclusions were similar.

We disagree with the interpretation of Shannon and Meeks-Wagner on only a few points but two of these warrant comment. First, they discuss *TFL1* function as if it were unique to and required solely for the maintenance of the inflorescence meristem. However, Tfl1 bolting occurs markedly earlier (with respect to both time and number of rosette leaves) than wild type. These data suggest that *TFL1* functions even during the vegetative phase promoting the formation of the inflorescence at the expense of the rosette. For these reasons we believe that the proposed COPS mechanism, in which *TFL1* plays a role, more accurately represents the available data.

Second, Shannon and Meeks-Wagner suggest that two separate pathways exist for FLIP; one represented by *LFY* and the other represented by *AP1* and *AP2*. In light of the additional data that we present, it is clear that all three genes act independently of one another and have differential roles. There seems no compelling reason to separate out *LFY* while grouping *AP1* and *AP2* together. Further, all three genes work toward a common end and are required for FLIP to function properly. Thus we prefer to group the three genes together. We believe that the evocation of separate pathways at this point is unnecessary and somewhat misleading.

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